A Summary of Current Program, 7/1/64,
and Preliminary Report of Progress
for 7/1/63 to 6/30/64

ANIMAL DISEASE AND PARASITE RESEARCH DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation, and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1963, and June 30, 1964. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

UNITED STATES DEPARTMENT OF AGRICULTURE
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INTRODUCTION

The Animal Disease and Parasite Research Division administers a national program of basic and applied research on diseases of cattle, poultry, swine, sheep, horses, and fur-bearing animals. The Division consists of three large laboratories and thirteen smaller, specialized laboratories. The large ones are the Beltsville Parasitological Laboratory, the National Animal Disease Laboratory at Ames, Iowa, and the Plum Island Animal Disease Laboratory at Greenport, Long Island, New York. The research at these locations cover, respectively, animal parasites, animal diseases existing in the United States, and foreign animal diseases. The smaller, specialized laboratories are located as follows:

The Southeast Poultry Research Laboratory, Athens, Georgia.
The Regional Animal Parasite Laboratory, Auburn, Alabama, with substations at Experiment, Georgia, and State College, Mississippi.
Fur Animal Disease Research Laboratory, Pullman, Washington.
Toxicological Research Laboratory, Kerrville, Texas.
Sheep Disease Research Laboratory, Denver, Colorado.
Poisonous Plants Research Laboratory, Logan, Utah.
The Florida Hog Cholera Research Laboratory, Live Oak, Florida.
Parasite Research Laboratory, Albuquerque, New Mexico.
Parasite Research Laboratory, University Park, New Mexico.
Parasite Research Laboratory, Tifton, Georgia.
Rabbit Experiment Station, Fontana, California.
Cooperative Research at the State Veterinary Research Institute, Amsterdam, Holland.
Cooperative Research at the East African Veterinary Research Organization, Kabete, Kikuyu, Kenya, East Africa.

In addition, the Division engages in cooperative research involving fifty cooperative projects at various Universities and State Experiment Stations. The Division's research program is coordinated by the Office of the Director, located at Beltsville, Maryland.

The Animal Disease and Parasite Research Division has contributed many significant research findings aimed at reducing the heavy losses to the livestock industry resulting from animal diseases. Several of these research discoveries have accounted for savings to the livestock industry in excess of the total cost of animal disease research in the U. S. Department of Agriculture since the inception of the Bureau of Animal Industry in 1887. Among these discoveries are the isolation and description of the genus of bacteria known as Salmonella; the role of arthropod vectors in spreading infectious diseases; the cause of hog cholera and the development of the first immunization procedure for this disease; the first successful treatment for hookworms in animals and man; the development of Strain 19 vaccine to prevent brucellosis, and the discovery of the cause of hyperkeratosis in cattle.

Some of the more recent accomplishments by this Division are -

Malformation in lambs and calves caused by poisonous plants. The conditions known as "monkey face" in lambs, and the so-called "crooked calf" disease were long thought to be inherited. Our scientists have shown that these conditions result from toxic fetal insult during early pregnancy. In the case of the "monkey face" in lambs, a cyclopian-type malformation, the toxic agent is the plant Veratrum californicum (false hellebore, wild corn, skunk cabbage). Ewes ingesting this plant on the 13th and 14th day of pregnancy produce malformed lambs. Continued ingestion beyond the 14th day causes an increased number of fetal deaths. In the case of the "crooked-calf" syndrome, the fetal insult is caused by wild lupines. This work is similar to, but pre-dates the discovery that the drug Thalidomide could cause malformed babies.

Advances in the Production of a Chemically Defined Medium for the Culture of Leptospira. For many years leptospira have been propagated in serum-enriched media. In order to standardize research and production procedures it is necessary to have chemically defined media. Leptospira pomona and 13 other serotypes have been successfully cultured through weekly transfers for two years in a medium composed primarily of an albumin supplement as oleic albumin complex and ammonium chloride. This is a step toward a better understanding of this bacterial pathogen of both man and animals.

Bovine Vibriosis. Researchers at the National Animal Disease Laboratory, reported that all heifers bred to an infected bull became infected at the first service. Only 2 of 12 infected heifers became pregnant as compared to 6 pregnancies in the 7 non-infected control heifers. The presence of a moderate inflammatory process in the uteri of the infected heifers suggested the underlying cause for the lack of conception.

Diagnosis of Hog Cholera. Since the discovery, in 1904, that hog cholera was caused by a virus, there has been a continuing effort to devise a laboratory diagnostic procedure for this serious disease. Recently a method of identifying hog cholera virus in animal tissues by the use of immuno-fluorescence has been developed. The virus is grown in swine kidney cells and stained with a fluorescent stain. It is then examined under a special microscope to demonstrate the virus-infected cells. This test has proved about 98 percent efficient in detecting hog cholera in experimental animals. The efficiency of this test in diagnosing field cases of hog cholera is being investigated. The test should be of great value in the campaign to eradicate hog cholera from the United States.

Biochemical Effects of Agricultural Chemicals. Research at the Division's Toxicological Research Laboratory at Kerrville, Texas, has shown that the feeding of vitamin A to cattle increased their susceptibility to poisoning by one of the systemic insecticides. This was particularly evident when cattle were also given phenothiazine drenches for internal parasite control. Four important enzyme systems were affected by the vitamin A-phenothiazine-

systemic insecticide combination. In another experiment, it was found that Brahman cattle were more susceptible to poisoning from two insecticides than were European breeds. This kind of research is helping to provide a fuller understanding of some of our pesticide problems in livestock.

Area Control of Hog Cholera with Inactivated Vaccines. Results compiled from a single county-wide pilot study area (Lowndes County, Georgia), using killed-virus vaccines, indicate that two 5-milliliter doses of vaccine, given one month apart, were effective in the control of hog cholera. Over a 2-year period (March 1962 to February 1964) approximately 60,000 swine were vaccinated in this manner. The killed-virus vaccines used had excellent immunizing properties. The advantage of killed-virus vaccine is that it is not a source of live virus which could be maintained in the pig for possible transmission to susceptible swine at a later time.

Drug-Resistance of Coccidia. Coccidiosis is the most important intestinal parasitic disease of poultry and is a serious disease in livestock. It has been found that the species of coccidia that cause cecal coccidiosis in chickens gradually acquired resistance to every drug tested. It was also found that in building resistance to one drug, the coccidia may also increase its resistance to another. This indicates that outbreaks of coccidiosis in flocks already receiving a drug will not be controlled by use of another drug. Development of such cross-resistance, however, doesn't necessarily work both ways. For example, coccidia exposed to one drug A, gain resistance to it and to an untried drug B, but if the coccidia are exposed initially to drug B, and develop resistance to it, they may remain susceptible to drug A until exposed to it long enough to build resistance. This work emphasizes the need for a continual search for new drugs for the control of coccidiosis.

The Survival and Inactivation of Foot-and-Mouth Disease Virus in Meat and Meat By-Products. In view of investigations it was concluded that foot-and-mouth disease virus may be present in the lymphatic system of vaccinated, and subsequently infected cattle. Presently available vaccination methods do not prevent the dissemination of foot-and-mouth disease virus through meat. Using seven known types of foot-and-mouth disease virus, it was shown that bovine lymph nodes contained virus as early as 12 hours, and as long as 15 days after inoculation. While considerable amounts of foot-and-mouth disease virus may be present in lymph nodes, it may be difficult to diagnose the disease by routine inspection procedures at the pre-clinical and convalescent stages of infection. Cattle slaughtered during the course of inapparent infection may disseminate foot-and-mouth disease through virus-infected animal products.

Foot-and-mouth disease virus was detected in joints of infected cattle and survived in synovial fluid of infected carcasses for 19 days when stored at 4°C. Virus remained infectious for several weeks in joints stored successively at chilling, freezing, and thawing temperatures. Foot-and-mouth disease virus was remarkably stable in blood and infected or contaminated animal tissues which had been spread on materials used to package

meat (wood, paper, metal). In several tests, the virus survived 48 days in blood spread on a can and stored at 4°C. These preliminary results indicated that meat-shipping containers may play a significant role in disseminating foot-and-mouth disease virus.

Research at Wisconsin has been carried out to determine how the environment which poultry are subjected to may influence their susceptibility to disease. This work has led to the discovery that levels of ammonia in air commonly found in poorly ventilated poultry housing renders birds more susceptible to infection with respiratory disease viruses than birds not exposed to this contaminated air. This work may explain why poultry disease problems such airsacculitis are much more prevalent under conditions of poor management.

Wisconsin and Utah workers have discovered that a condition of sheep previously of unknown cause and referred to by livestockmen as "stiff lamb disease" is caused by a virus. The disease is now known as "Polyarthritis." Utah has found that Polyarthritis is widely distributed in sheep in the Western Mountain area and can cause serious outbreaks of a similar disease in calves. Workers are now concentrating on efforts to provide methods for Polyarthritis control in sheep and calves.

Recent research at a number of State stations has shown the value of a drug known as Thiabendazole as a treatment for livestock parasites. Kentucky workers have now found evidence that certain parasites may develop a tolerance for this drug after several repeated exposures. This finding emphasizes that indiscriminate use of thiabendazole must be avoided if maximum effectiveness is to be obtained.

Much progress is being made in determining causes of reproductive diseases in cattle. California, Colorado and Ohio workers recently have discovered that outbreaks of abortion can be caused by infectious bovine rhinotracheitis - a virus disease which previously was thought to affect only the respiratory tract of cattle. Connecticut research has found some cases of bovine sterility to be due to infection with Mycoplasma - an organism with bacterial and viral-like characteristics. Severe outbreaks of mastitis also were traced by these workers to a similar Mycoplasma agent.

The control and eventual eradication of animal diseases depends upon intensive research, both fundamental and applied. This research can be done adequately only by highly trained specialists supplied with the most modern research laboratories, equipment, and large animal isolation units. A start has been made in this direction, but if we are to meet the present needs and those of the future, a much greater support for animal disease research is mandatory.

AREA NO. 1 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF CATTLE

Problem. Losses from infectious and non-infectious diseases of cattle, other than those due to parasites, are estimated at approximately $600 million annually. These losses materially increase costs of production and conversely decrease profits. In turn, they contribute to the cost of every purchase of meat, milk, and other cattle products to the consumer. Some of these diseases are transmissible to man. Determination and definition of the causes of cattle diseases, explorations for efficient methods of diagnosis, prevention, control, and when feasible, eradication, are the purposes of the research program.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of cattle. Research is being conducted on the diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 57.7 professional man-years. This effort is divided among sub-headings as follows:

Brucellosis of Cattle 2.3 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the University of Minnesota, the University of Wisconsin, and with the Ohio Agricultural Experiment Station. A project on the immunizing effect of Brucella cell wall is in progress at the Hebrew University, Jerusalem, Israel, under a PL 480 Grant of funds equivalent to $31,950.00 over a 3-year period.

Vibriosis of Cattle 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the New York State Veterinary College at Ithaca.

Tuberculosis of Cattle 6.6 at the National Animal Disease Laboratory, Ames, Iowa, and through two contracts with the Michigan State University at East Lansing.

Mucosal-Respiratory Disease-Complex 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Colorado State University at Fort Collins, the Agricultural Experiment Station, Purdue University at Lafayette, Indiana, and the Iowa State University, Ames.

Mastitis of Cattle 6.2 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Respiratory Disease of Cattle (Shipping Fever) 5.0 at the National Animal Disease Laboratory, Ames, Iowa.

Leptospirosis of Cattle 6.0 at the National Animal Disease Laboratory, Ames, Iowa.

Infertility in Cattle, other than Vibriosis and Trichomoniasis 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Epizootic Bovine Abortion 3.4 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the Agricultural Experiment Station at Ames.

Foot Rot (Infectious Pododermatitis) of Cattle 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Etiological, Cytological and Histochemical Studies of Pulmonary Adenomatosis in Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Immunization Against Bovine Leptospirosis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Chemotherapy in Leptospirosis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Nature and Immunogenicity of Leptospiral Lipids 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Paratuberculosis of Cattle (Johne's Disease) 5.0 at the National Animal Disease Laboratory, Ames, Iowa.

Keratitis (Pink Eye) 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

PROGRAM OF STATE EXPERIMENT STATIONS

The State experiment stations are active in conducting basic and applied research pertaining to the prevention, control and eradication of diseases of cattle. Objectives of these studies not only concern the health and well-being of animals but also reflect the increasing interest in the role of diseases of animals to the health of human beings. Research workers are concerned in delineating the cause of specific conditions, developing techniques for the improvement of diagnoses, finding new methods of increasing resistance to disease and/or decreasing the exposure to infectious agents.

Factors which affect the immune response in vaccinated calves and the development of new tests to increase the speed and accuracy by which brucellosis-infected animals can be detected are under investigation.

Cooperative regional studies among the Northeastern (NE-40, Pathology of Breeding Failure) and Southern States (S-30, Diseases of Reproduction) seek to determine the relation of infectious agents to poor reproductive performance and sterility in cattle. Antigenic variations in strains of the organism causing vibriosis are being studied to improve diagnostic techniques and to develop possible immunizing agents. The role of leptospira in infertility is being determined and detailed studies on the pathology produced by different serotypes of the organism are being elucidated.

Many of the North Central States are cooperating informally (NCR-37), Mucosal Disease; NCR-29, Shipping Fever) to determine the causes of bovine respiratory problems and to develop methods for control. Preventive vaccines are being developed and evaluated under laboratory and field conditions. The relation of infectious bovine rhinotracheitis to the respiratory disease complex is also being investigated.

Studies seek basic information pertaining to the cause of mastitis and the fundamental factors that influence resistance of individual cows. Prophylactic and therapeutic agents are being studied to evaluate their efficacy and milk residue properties.

Workers in many States are studying the interrelationships between various agents and factors associated with intestinal infections in cattle, particularly those causing severe losses in newborn calves.

Attempts are being made to clarify the cause of foot rot and infectious keratitis or pink eye. There is some evidence that viral agents may be responsible for these conditions.

Much attention (Regional Research Project, W-41, Urinary Calculi of Beef Cattle) is being given to possible factors which lead to the development of urinary calculi of cattle. Consideration is being given to the theory that an imbalance of certain nutritional elements may contribute to the development of the condition.

New diseases are being encountered constantly and diseases not previously encountered or not regarded as a problem, often become economically important enough to require intensive study. Other bovine disease problems being investigated currently include the various abnormalities, malignant lymphoma, tuberculosis, paratuberculosis, epizootic abortion, ketosis, parturient paresis, white muscle disease, aplastic anemia, enterotoxemia, etc.

The total State scientific effort devoted to diseases of cattle is 52.8 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Brucellosis of Cattle

Research work conducted at the National Animal Disease Laboratory (NADL), Ames, Iowa, was reported as follows:

1. Pathology: a) Two bulls, naturally infected with Brucella abortus were studied for 5 and 2 years, respectively. Serologic, bacteriologic and histopathologic examinations were correlated with the clinical signs of the disease. Seroagglutinin and semen plasma agglutinin titers persisted at diagnostic levels throughout the study, and Brucella abortus was consistently isolated from the semen of both bulls. At necropsy Brucella abortus was isolated from the testes, epididymides, seminal vesicles and the ampullae of the ductus deferens. Pathologic changes were observed throughout the genital tract. Granulomas, including sperm granulomas, were found in the epididymis of one bull.

b) In two other bulls infected with brucellosis, the etiologic agent was Brucella abortus Strain 19. One bull, vaccinated at 6 months of age developed a bilateral orchitis within 10 days. Two months postvaccination a bilateral castration was required. A second bull was vaccinated at 5 months of age. Eight months later the seroagglutination test showed the bull had a titer of +50. The semen plasma test titer was +400. Brucella abortus Strain 19 was readily isolated from the ejaculate of each bull. At necropsy Strain 19 was isolated from the seminal vesicles, prostate, urethra and the epididymides. Pathologic alterations were primarily confined to the accessory genital organs and semen quality was not noticeably affected. The necessity and wisdom of employing the semen plasma agglutination test in addition to the blood serum tests was clearly indicated as a means of detecting potential spreaders of brucellosis.

2. Serology: a) Nonspecific agglutinins for Brucella were isolated from 7 cattle by techniques for absorption of serum by Brucella cells and differential ultracentrifugation. The agglutinins had high molecular weights with sedimentation coefficients varying from 13.8 to 16.6 Svedburg units. The isoelectric points of the purified agglutinins determined by microelectrophoresis correlated positively with the heat stability of the seroagglutinins. With one exception the heat stable (56 C, 18 hr.) agglutinins had an isoelectric point of pH 4.7, whereas the heat labile agglutinins had an isoelectric point of pH 4.3. The activity of the purified agglutinins ranged from 0.1 to 0.5 ug of protein per unit of agglutinin. The ultraviolet absorption maximums of the agglutinins fell into the range of typical proteins.

b) These physicochemical studies were continued on Brucella agglutinins produced by heifers after vaccination with Brucella abortus Strain 19. One week after vaccination all agglutinins were of high molecular weight. In the second week postvaccination some low molecular weight seroagglutinins

were detected. The high molecular weight agglutinins reached a maximum concentration approximately 13 days postvaccination. Low molecular weight agglutinins reached a maximum at 28 to 42 days postvaccination. With few exceptions the fast sedimenting agglutinins predominated throughout the 91-day study. There was a positive correlation between the percentage of heat labile (65 C, 15 minutes) agglutinins and the percentage of fast sedimenting agglutinins. The percentage of agglutinins inactivated by mercaptoethanol was closely related to the percentage of fast sedimenting agglutinins.

c) A continuation of this research involved density gradient ultracentrifugation and heat stability (65 C, 15 minutes) studies on Brucella seroagglutinins of pregnant heifers artificially infected with virulent Brucella abortus. During the first two weeks postexposure, all of the agglutinins detected were high molecular weight type. Low molecular weight agglutinins were first detected between the 15th and 29th days postexposure. As infection progressed, the concentration of slow sedimenting (low molecular weight) agglutinins became equal to and then exceeded that of the high molecular weight agglutinins. As in the former studies there was a high positive correlation between the percentage of heat labile agglutinins and the percentage of high molecular weight agglutinins in each serum.

3. Immunology

Eighteen vaccinated and 5 nonvaccinated heifers in midgestation were exposed to virulent Brucella abortus strain 2308. Changes in their serum proteins were studied by paper electrophoresis for 29 weeks postexposure. In the serums of the heifers that became infected (4 vaccinated and 4 nonvaccinated), the relative percentage of gamma globulin over albumin was greater and persisted longer in the serums from infected nonvaccinated than in infected vaccinated heifers. Changes in the amount of gamma globulin roughly paralleled the changes in the seroagglutinin titers. Only minor changes occurred in the concentrations of albumin and globulin in the serums from 14 vaccinated and one nonvaccinated heifer that did not become infected. (Iowa) (ADP al-3(Rev.)

The University of Minnesota, under a cooperative agreement with the USDA, reported the studies during the past year were concerned with the study of physico-chemical characterization of antibodies for Brucella found in milk and serum of cattle and swine, and the development of methods to separate the several classes of antibodies for Brucella found in bovine milk.
(Minnesota) (ADP al-3(Rev.)

The University of Wisconsin, under a cooperative agreement with the USDA, reported work on a method of standardizing the complement-fixation (CF) test for bovine brucellosis, utilizing the Brucella antigen for the standard serum agglutination tube test. With the International Standard for anti-Brucella abortus serum, the method compared favorably in sensitivity with methods used in European laboratories.

Over 1400 serum samples from cattle in brucellosis problem herds in Wisconsin were examined by the standard serum agglutination tube test and the CF test. The CF test was a useful supplemental test for serums with suspect titers to the agglutination test. In several herds in which infection was recent, cattle developed CF titers before they became agglutination reactors. (Wisconsin) (ADP al-3(Rev.)

Research work was initiated at the Ohio Agricultural Experiment Station, Wooster, under a cooperative agreement with the USDA. The first phase of the study on early vaccination of calves against Brucellosis was completed. Calves 2 and 3-months of age, respectively, were tested by serological techniques and then vaccinated with Brucella abortus, Strain 19. (Ohio) (ADP al-3(Rev.)

Investigations on "The Immunizing Effects of Brucella cell wall" are in progress at the Hebrew University, Jerusalem, Israel, under a PL 480 Grant (A10-ADP-6). The preliminary work, using experimental animals, has been mainly of a confirmatory nature. However, it appears encouraging. (Israel)

B. Vibriosis

Research conducted at the National Animal Disease Laboratory, Ames, Iowa, was reported as follows:

1. Reproductive Patterns of Vibrio fetus-infected Cattle. Work has been completed on a study of the breeding patterns of 31 female cattle bred for 1 to 4 successive calf crops to Vibrio fetus-infected bulls. Twenty-eight of these cows became infected at first service to an infected bull. One of the remaining 3 became infected at first service for her second calf; one became infected at second service to an infected bull, and the other did not become infected when bred for 2 pregnancies.

The average duration of infection with V. fetus was 180 days, ranging from 14 to 313 days. All except 1 cow recovered spontaneously between gestation periods and remained free of infection until they were rebred, at which time 60% became reinfected. The exception was one heifer which remained infected during gestation and thereafter until necropsy, 66 days after calving, when V. fetus was isolated from her uterus.

The cows required more services and more time from first service to pregnancy when first infected than did those which were reinfected; however, some reinfected cows also remained infected throughout subsequent gestation periods.

This study indicates that although immunity was not established with first infection, heifers artificially exposed with V. fetus at sexual maturity might be stimulated to produce resistance before service for their first calf crop and thus breed satisfactorily without significant lost time.

2. Vibrio Infection of the Digestive Organs of Cattle. Eighteen cattle were orally inoculated with broth suspensions of Vibrio fetus type 1, subtype 1, and type 2 to study the infectivity of each for the digestive organs. Six of 7 cattle fed type 2 became infected and shed the organism in their feces for variable periods. One cow remained infected for 4 weeks. She became reinfected after feeding thie type again and remained infected for 16 additional weeks. Another cow, infected for 6 weeks, did not become reinfected when inoculated a second time. Neither type 1 nor subtype 1 V. fetus infected any of 11 cows inoculated. At necropsy type 2 V. fetus was isolated from the duodenum, bile, bile duct, liver, and pancreatic duct of cattle up to 5 months after feeding.

It was apparent from this study that only type 2 V. fetus infects the digestive organs of cattle and although type 1 and subtype 1 proliferated in the reproductive organs of cattle and caused repeat breeding, they are unable to live in the digestive organs. Type 2 has been considered an intestinal inhabitant which has the capacity to cause sporadic abortion in cattle and sheep.

3. Fluorescent Antibody Studies. Fluorescent antibody conjugates capable of producing fluorescence in V. fetus cells were prepared by several methods. It appears that the success of fluorescent staining varies with the method of serum fractionation employed. The performance of conjugates prepared from serum fractionated with ammonium sulfate was superior to that of other conjugates. Bright staining was observed more frequently with bovine serum conjugates than with conjugates of rabbit origin. While the staining of cell suspensions was rapid and simple, better results were obtained by staining smears. (Iowa) (ADP al-9(Rev.)

The New York Veterinary College, Cornell University at Ithaca, under a cooperative agreement with the USDA, continued research studies on diagnostic procedures for vibriosis. The following findings were reported:

A. Incidence of vibriosis in an artificial insemination stud. From 1952 to July 1963, 12,644 semen samples from 432 bulls were cultured for Vibrio fetus. Analysis of the data indicated that 20.4 percent of all bulls examined were carriers of V. fetus. There was a highly significant age effect on the incidence of V. fetus. Of 233 bulls under 6 years of age, 4 (1.7 percent) were carriers, whereas 65 (46.7 percent) of 139 bulls 6 years of age or older were carriers.

B. Diagnosis of vibriosis in the bull by use of fluorescent antibody technique. The objective of this project was to develop a fluorescent antibody technique for diagnosing vibriosis in bulls. Although culture techniques for recovering V. fetus from bulls have been improved during the last few years, limited numbers of culture attempts cannot be relied upon for detecting all carrier bulls. The use of virgin heifers as test animals is more accurate, but is too expensive for routine use.

The fluorescent antibody technique has been successfully adapted for diagnostic purposes by using a conjugate purified to eliminate most of the non-specific staining reactions and by concentrating the organisms in samples of sheath scrapings through centrifugation. On the basis of present results, it appears that this is a more sensitive method of diagnosis than the best culture methods and that it probably ranks with the heifer-mating test in its efficiency. (New York) (ADP al-9(Rev.)

C. Tuberculosis

Research was continued at the Michigan State University under two contracts with the USDA. Reports submitted are as follows:

(Contract No. 12-14-100-6852(45). Lipids extracted by ether-ethanol from 25 strains of mycobacteria were fractionated by absorption chromatography. Infrared spectra of the fractions were recorded. Type-specific lipid compounds were found in the extracts of human, bovine, avian and atypical strains.

Demoycoceronate of phthioceral was found in the lipids of two human strains ($H37R_A$ and $H37R_V$) and one bovine strain (M. bovis Ravenel), mycoside B was isolated from M. bovis Ravenel but not from M. bovis B.C.G. Other type-specific lipids found were: mycoside A isolated from two photochromogens (P-4 and P-8), mycoside F from M. fortuitum, mycoside C from M. avium and 158 C-0 (isolated from a bovine mesenteric lymph node), mycoside D from 71C-0 (also from a bovine mesenteric lymph node) and mycoside C_M from strain P-31 and 12 organisms isolated from swine mesenteric lymph nodes and bovine body lymph nodes and Peyer's patches.

(Contract No. 12-14-100-7164(45). This contract was initiated during the reporting period. Experiments are in progress. Cattle were obtained which were not sensitive to tuberculin by caudal fold and cervical tests. The necessary facilities have been obtained to permit studies on chromatograph. (Michigan)

Contract No. 12-14-100-5786(45), on the Role of Heat-Killed Mycobacteria and Feed Supplements of Animal Origin in Producing Tuberculin Hypersensitivity in Cattle, was completed, and the researchers at the Michigan State University submit the following summary of their findings:

Sixty nonpregnant predominantly Guernsey crossbred heifers were fed one of four different rations for 160 days to determine if any of the rations would induce delayed hypersensitivity in the animals as detected with 0.1cc mammalian tuberculin injected intradermally. The animals were obtained from a herd with no tuberculin reactors and had no detectable response to mammalian or avian tuberculins or johnin when tested in the caudal fold and cervical regions. They were maintained during the study in four isolated groups of 15 each. The control group was fed a ration in which the protein concentrate was soybean oil meal and the mineral concentrate was dicalcium phosphate. The second and third groups were fed the control ration to which daily was added $5 \times 10^{8-9}$ heat-killed (121C moist heat for 30 minutes) Mycobacterium bovis and Mycobacterium avium, respectively. The fourth group was fed a ration in which the protein concentrate was meat and bone scrap and the mineral concentrate steamed bone meal.

Tuberculin tests using 0.1 cc mammalian tuberculin were performed on all animals at three different times. Some were tested at 20, 30 or 40 days, and all were tested at 100 and 160 days following the start of feeding the experimental rations. No animal was classified as a reactor at the official reading time. (Michigan) (ADP al-13(Rev.)

D. Mucosal-Respiratory Disease-Complex of Cattle

Research studies were continued at the National Animal Disease Laboratory, Ames, Iowa. Reports submitted showed that calves inoculated with bovine viral diarrhea (BVD) viruses and soluble antigen, the complement-fixing (CF) antibodies appeared before serum-neutralizing (SN) antibodies and remained at high levels throughout the test period. A rapid rise in SN antibodies occurred after challenge with homologous virus with no apparent effect on CF antibody levels.

The CF antibody responses in calves infected with cytopathogenic NADL-MD and noncytopathogenic CG-1220 viruses were similar, whereas SN antibody responses indicated strain specificity by reciprocal cross-neutralization tests.

The CF antibody levels in 5 hog cholera (HC) antisera were assayed, using the soluble antigen of NADL-mucosal diarrhea-bovine virus diarrhea virus. No demonstrable SN antibodies were present in four HC antisera tested against NADL-MD virus, but a significant titer was present in the commercially prepared antiserum.

Virus was reisolated from animals infected with BVD viruses by buffy coat culture technique during 3 weeks' postinoculation, even when significant levels of CF and SN antibodies were present.

Noncytopathogenic (NCP) bovine viral diarrhea disease agents can be detected and titrated in tissue culture systems by a method employing immunofluorescence. Cytopathogenic (CP) and non-CP (NCP) viruses cross-react with fluorescein-conjugated serum globulins produced against either CP or NCP viruses, but the fluorescence is more intense in the homologous serum. Serum neutralization titers of sera against both CP and NCP groups were compared for both groups of viruses, and results of cross reactions were in agreement with results from immunofluorescence tests. (Iowa-NADL)

Colorado State University, Fort Collins, under a cooperative agreement with the USDA, reported that during the past year the serum neutralization titer of the cattle which were kept in the isolation units did not show lowering of titer. There were 3 lots of cattle with 4 animals per lot. One group was injected intratracheally, one intramuscularly, and the third lot served as control. There was no difference of titer between the two infected lots.

Pathological studies of infectious bovine rhinotracheitis in relation to abortion are being conducted using 35 virgin heifers that were negative to serological tests for this disease, brucellosis, and leptospirosis. Progress is being made on this phase of the research. (Colorado)

Research work conducted at Purdue University, Lafayette, Indiana, under a cooperative agreement with the USDA, was a continuation of tissue culture, fluorescent antibody, and serological investigations.

Sporadic cases of the mucosal disease complex continue to occue in Indiana. The apparent incidence of this disease complex has not changed from previous years.

Cytochemical and cytological studies on the growth of Oregon $C24_V$ virus in tissue culture were made. The application of the acridine orange (AO) staining procedure to infected lamb thyroid cultures gave evidence that $C24_V$ virus is of the RNA type. Furthermore, AO and phase microscopic studies suggest that replication takes place in the cytoplasm of infected cells. The data derived from these and other growth studies will be utilized in applying fluorescent antibody procedures for detection of virus diarrhea-mucosal disease agents in clinical specimens and tissue culture systems.

On initial passage $C24_v$ virus was capable of producing cytopathic changes in cultures of bovine and ovine kidney, testicle and thyroid tissues. In an explant-type culture system employing lamb kidney tissue, cytoplasmic inclusion-like lesions were observed. The development of cytoplasmic inclusions and the general cytopathic effects of virus were inhibited by specific immune serum. Further study is needed to determine the specificity of the cytoplasmic lesions observed.

Two virus isolations made from field cases of "mucosal disease" were grown on bovine embryonic kidney and lamb thyroid cells. Serums from Specific-Pathogen-Free calves recovered from experimental infection with the two field isolates neutralized Oregon $C24_v$ in tissue culture tests. The new virus isolates appear to be immunologically and serologically related to other virus diarrhea-mucosal disease viruses.

The specific-pathogen-free (SPF) cattle herd continues to be relatively free of important pathogens. The reproductive efficiency of the herd is normal and about 24 calves will be available for research during the next twelve months. (Indiana)

At the Iowa State University during the past year, research results have pointed to the fact that both viral diarrhea and infectious bovine rhinotracheitis may elicit a clinical and pathological syndrome which is indistinguishable. They have verified this fact by fluorescent antibody staining of viral antigen associated with Herpes-virus-induced lesions. Results further indicate that the entire group of enteroviruses may be excluded from the viral diarrhea problem in cattle, but play an important role in enteric problems of young calves. (Iowa State Univ.)(ADP al-14C(R)

E. Mastitis of Cattle

The research studies at the National Animal Disease Laboratory, Ames, Iowa, pertained to the following:

1. Three cultures of group A hemolytic streptococci have been serially subcultured for an extended period (100 or more serial transfers) in a peptide- and protein-free medium. In 24-48 hours incubation at 37°C, luxuriant growth was obtained with complete removal of 1 percent glucose and quantitative fermentation to lactic acid. Optical densities of cultures were 0.40 - 0.50. An amino acid assay medium, modified by addition of small amounts of glutamine, ammonium acetate and 0.1 M phosphate, pH 7, was used. In this medium high concentrations of glutamic acid or glutamine were required and biotin was stimulatory to growth. Biotin could be partially replaced with $NaHCO_3$. Maximal growth was obtained with $NaHCO_3$ when biotin was present and aspartic acid and asparagine were omitted from the medium.

2. Three strains of Streptococcus pyogenes, Richards (type 3), N19 (type 19) and S43 (type 6), after repeated subculturing in a chemically defined medium (100 or more times), were each tested for the group-specific and type-specific antigens by the precipitin test. On three separate tests for each culture, the group A antigen ("c" polysaccharide) was present, but the type-specific antigen (M-protein) was absent. The same strains grown on a chemically defined medium containing reduced ovalbumin showed no loss of M protein. (Iowa - NADL)

The University of California, Davis, under a cooperative agreement with the USDA, reported that the results of several years of investigation indicate that coliform mastitis is a disease of the normal lactating mammary gland. It was concluded that the relative infrequency of occurrence of coliform mastitis, in commercial dairy herds, is due to the presence of a leukocyte barrier in lactating glands of older cows. This leukocyte barrier is in response to the stress on mammary tissues of modern methods of mechanical milking and bacterial infection with common udder pathogens.

To further substantiate the role of the leukocyte in controlling multiplication of coliform bacteria within the udder, an attempt was made during the current year to delay the infiltration of leukocytes into exposed mammary glands. To this end, corticosteroids, both intramammarily and systemically were employed. Selection of corticosteroids for this purpose was based on the claimed ability to inhibit diapedesis of leukocytes into foci of developing inflammation. The corticoid employed was 9α fluoroprednisolone acetate (Upjohn) at dose levels of 50 mg. to 1,000 mg. per cow. Such doses were greatly in excess of the quantities commonly incorporated in antibiotic preparations for therapeutic treatment of mastitic glands. Administration of the corticoid prior to and simultaneously with coliform bacteria failed to delay or reduce the magnitude of the leukocytic infiltration. Despite the mobilization of circulating neutrophil leukocytes to levels up to 6 times normal following systemic application of the corticoid, the leukocytic activity within the mammary gland exposed to coliform bacteria was not enhanced. Escherichia coli and Aerobacter aerogenes are not considered true pathogenic bacteria. Clinical disease is produced by the release of endotoxin when the bacterial cells of a massive population are destroyed by leukocytic action.

In order to determine if a leukocyte barrier can exist for recognized pathogens of the mastitis complex, attention was directed toward Streptococcus agalactiae. Through natural selection this organism has become an obligatory parasite of the mammary gland. Its potential for establishment of an enduring infection within the mammary gland is well known. Four lactating heifers and 5 older cows, none of which had any previous exposure to Str. agalactiae were available. Pre-existing leukocytic infiltration into mammary quarters were of natural or experimental origin for investigation of the leukocyte barrier. An adequate number of quarters secreting cell-free milk was available for controls. Among 16 glands serving as control and receiving a single exposure to Str. agalactiae at levels of

5 to 600 colony-forming units, infections were established in 6 glands (37%); among 7 control glands exposed 3 times at 12-hour intervals to between 6 and 400 Str. agalactiae, the infection rate was 50 percent. Thus, it was established that small numbers of Str. agalactiae are potentially capable of establishing infection in essentially normal lactitating quarters.

Failure of Str. agalactiae to establish itself within an exposed lactating mammary quarter may be in consequence of one or more or a combination of the following:

1) Pre-existing leukocytic infiltration at levels serving as a barrier per se to Str. agalactiae multiplication.

2) Capability of a gland to infiltrate millions of leukocytes within the first few hours after introduction of Str. agalactiae. Glands previously injured but having returned to low infiltrating cell numbers appear to be uniquely capable of immediate mobilization of large numbers of leukocytes.

3) Participation of humoral factors from infection of opposite quarters with Str. agalactiae and, perhaps, other streptococci.

Experiments on the endotoxin of A. aerogenes confirmed a previous hypothesis that the clinical signs of A. aerogenes peracute mastitis were probably referable to the release of endotoxin following lysis of acteria by the inflammatory exudate. Endotoxin at levels of from 0.2 mg to 20.0 mg produced the same array of signs and symptoms as those seen following unlimited growth of A. aerogenes. The endotoxin had all the characteristics of endotoxin from R variants of gram negative bacteria. Trials in cats and a horse showed cats to be rather resistant to the endotoxin, whereas one horse died within 9 hours following administration of a safe dose calculated from mouse inoculation data.

The probable identity of the protein "X" with paper electrophoretic mobility intermediate between α-lactalbumin and immune globulin that appears in whey following agalactia of mastitis or in early dry cow secretion was confirmed. The protein when isolated by preparative electrophoresis does not have the same mobility in the isolated state but behaves as immune globulin. The isolation procedure is considered to be sufficiently mild that denaturation was not involved. (California) (ADP a1-15(Rev.)

F. Respiratory Diseases of Cattle (Shipping Fever)

Research investigations conducted at the National Animal Disease Laboratory, Ames, revealed that smooth Pasteurella haemolytica, after rapid growth in statically incubated broth cultures, decreased in numbers rapidly, and were replaced by nonsmooth variants. Upon continued incubation, smooth cells again predominated. The two phenotypes were alike in general biochemical characteristics, but differed in virulence for mice. The presence of non-

smooth cells in mixed cultures limited the growth of smooth cells. The inhibition of smooth cells correlated with the establishment of definite population densities, and the critical factor was limitation of oxygen in the cultural medium. Selective inhibition did not occur in aerated cultures, but was more pronounced in cultures grown under reduced air pressure. Selective death of smooth cells on slant cultures held at 5°C, and preferential growth of nonsmooth cells, plus death of smooth cells at room temperature, accounted for population changes in stored cultures.

Bovine parainfluenza-3 (PIV-3) virus was isolated from nasal mucus of cattle with signs of shipping fever by amniotic inoculation of 14-day-old embryonated hen's eggs. Virus isolated in this manner could not be demonstrated in the amniotic fluid, but after 3 passages in the amnion could usually be recognized by agglutination of guinea pig erythrocytes. Primary virus isolates could, however, be recognized by inoculation of embryonic bovine kidney (EBK) cells. Multiplication of PIV-3 virus in the embryonated hen's egg did not result in death of the embryo.

Virus isolations from diluted specimens suggested that the chick embryo is more susceptible to PIV-3 virus infection than are tissue cultures of EBK cells. Egg inoculation also permitted the selection of PIV-3 virus in the presence of infectious bovine rhinotracheitis virus which was demonstrated in several samples. Attempts to adapt virus isolated in the amnion to the allantoic cavity of younger embryos were not successful. (Iowa-NADL)

(ADP a1-17)

G. Epizootic Bovine Abortion

The University of California, under a cooperative agreement with the USDA, reported the following:

1. Studies to be terminated this year indicate conclusively that vaccination with a modified live virus vaccine prepared from an agent (Miyagawanella felis) related to the virus of epizootic bovine abortion (EBA) is ineffective in preventing abortion due to the EBA virus. The current approach to preventive immunization is by means of a vaccine consisting of an attenuated strain of EBA virus, given when heifers are six months of age and repeated just prior to breeding. Attempts to attenuate the virus are currently in progress.

2. In view of indications that EBA is a venereally-transmitted infection, studies designed to determine the validity of this observation are under way. Should this mode of transmission be proved, artificial insemination is regarded as the ultimate solution to the problem of prevention and control.

3. Progress in serological studies relative to the EBA virus has been made.

4. Preliminary findings suggest that abortion due to the virus of infectious bovine rhinotracheitis (IBR) is restricted to those strains of virus which have acquired an enhanced invasiveness for the blood stream of cattle. This property appears to have been acquired fairly recently as studies of early respiratory isolates of the virus indicate that such strains lack this characteristic.

5. The virus of enzootic abortion of ewes (EAE) has been isolated in ewes in California and Oregon. This represents the first isolation of this virus outside the enzootic areas of Montana, Idaho, and Utah. (California) (ADP a1-21)

H. Immunization Against Bovine Leptospirosis

The National Animal Disease Laboratory, Ames, Iowa, reports that Leptospira pomona and 13 other leptospiral serotypes, were subcultured at weekly intervals for 2 years in a medium primarily composed of Oleic Albumin Complex and NH_4Cl. The albumin functioned as a detoxifier of oleic acid and as a source of nitrogen because continuous subculture was possible without adding NH_4Cl, but at a markedly reduced level of growth. Added vitamin B_{12} was required for growth of representative members of each serotype studied. Additions of NaCl stimulated growth.

A commercial complex of bovine albumin and oleic acid (OAC), which replaced whole rabbit serum in leptospiral medium, was fractionated. The growth-supporting function of each fraction was studied, and the fractions were replaced with specific nutrients. Basal medium supplemented with bovine albumin and sodium oleate or Tween 80 supported good growth of 14 leptospiral serotypes through indefinite subcultures with undiminished growth and unaltered antigenicity.

Oleic albumin complex was extracted with ether. The ether extract, when recombined with extracted OAC, supported good growth. Alkalinized oleic acid, sodium oleate, or Tween 80 satisfactorily supplemented several albumins of bovine origin. Adding lipid to spent medium restored its growth-supporting capability. If Tween 80 was used, 0.5% albumin was adequate for cultivation of Leptospira pomona.

Leptospira grippotyphosa was isolated from the urine of a cow 7 days after abortion. The isolant grew poorly in Stuart's liquid medium and Fletcher's semisolid medium. Experimental semisolid and liquid media, containing bovine albumin fraction V and Tween-80, proved valuable as isolation and growth media. Gerbils and hamsters were more susceptible than guinea pigs and white mice to the newly isolated organism. Serological evidence indicates that L. grippotyphosa is widely distributed in Illinois cattle and swine. (Iowa-NADL) (ADP a1-25)

I. Chemotherapy in Leptospirosis

Investigations at the National Animal Disease Laboratory, Ames, Iowa, have determined the effects of certain antibiotics and polylysine on leptospirae in synthetic medium and medium supplemented with rabbit serum. No differences in sensitivity to antibiotics and polylysine were found among cultures of Leptospira pomona, L. canicola, L. autumnalis, and L. grippotyphosa in synthetic medium. All the antibiotics tested were leptospirastatic in low concentrations. Tylocin and erythromycin were effective in the lowest concentration (0.025 µg/ml) and sterilized cultures the quickest (72 to 96 hours); chlortetracycline and oxytetracycline (0.5 µg/ml) prevented multiplication but failed to sterilize cultures in 10 days. Little or no leptospiral immobilization was observed in cultures containing bacteriostatic levels of antibiotics; most antibiotics lysed leptospirae at ten times the leptospirastatic concentration. In Stuart's medium containing 10% rabbit serum, penicillin and oxytetracycline were two and four times less effective than in synthetic medium, respectively. (Iowa-NADL)
(ADP al-26)

J. Nature and Immunogenicity of Leptospiral Lipids

Research workers at the National Animal Disease Laboratory, Ames, Iowa, reported that Leptospira canicola cells were grown in a chemically characterized medium containing Tween 80. Lipid extracted with chloroform: methanol (2:1) from washed, lyophilized cells was equivalent to 16% of the dry cell weight. Approximately 50% of this lipid was present in the phospholipid fraction.

Fatty acids from whole cells were tentatively identified by gas-liquid chromatography of the methyl esters using diethylene glycol succinate and Apiezon L as liquid phases. Unsaturated esters were removed as mercuric acetate adducts.

Of the dialyzable lipid, octadecenoic acid was the major acid accounting for 47% by weight of the fatty acids. Hexadecanoic acid accounted for 19% of the fatty acids. The next largest component (9%) was an unidentified, unsaturated fatty acid with a carbon number of 15.25 on the DEGS polyester column. The other acids listed in descending order of abundance were: an unidentified saturated acid with the same retention volume as a 17-carbon branched-chain acid, hexadecenoic acid, octadecaienoic acid, tetradecenoic acid, an unsaturated acid with a carbon number of 12.75, octadecanoic acid and traces of octanoic, tetradecanoic acid and several other unidentified acids. Acids with retention volumes corresponding to 17 or 19-carbon cyclopropane fatty acids were not noted. (Iowa-NADL) (ADP al-27)

K. Paratuberculosis (Johne's Disease) of Cattle

The National Animal Disease Laboratory, Ames, Iowa, reported that experiments were conducted to find a combination of decontaminant and medium that would be more satisfactory for the primary cultivation of Mycobacterium paratuberculosis. Sodium hydroxide, sodium hypochlorite, phenol, and benzalkonium chloride (Zephiran) were compared as decontaminants, and specimens treated with these agents were cultured on lymph-node-egg-yolk medium and modified Herrold's medium (an egg-yolk-agar medium containing mycobactin). The most satisfactory combination was decontamination with benzalkonium chloride, followed by inoculation onto modified Herrold's medium. This technique allowed the demonstration of Myco. paratuberculosis in tissues in which the organisms were present in such small numbers that they could not be found by microscopic examination.

Blood samples were obtained periodically for complement-fixation tests from all cattle in a herd of 161-195 where Johne's disease has been an economic problem. Selected tissues of all cattle removed from the herd were examined for Mycobacterium paratuberculosis after slaughter.

Observations were made on 93 cattle eliminated from the herd during a 5-year study. Fifty-four cattle had titers of 1:32 or more; 12 of these developed clinical evidence of Johne's disease, and 23, including the aforementioned 12, harbored M. paratuberculosis. Thirteen of the remaining 39 cattle with serum titers of 1:16 or less harbored the bacillus at slaughter, and 3 of these had developed clinical evidence of Johne's disease. Forty cattle were tested 6 times in 2-1/2 years, a total of 240 tests; titers remained constant or changed only 1 dilution in 185 instances. It increased 2 dilutions in 14 instances, 3 dilutions in 6 instances, 4 dilutions in 5 instances, and 5 dilutions in 1 instance. The titer decreased 2 dilutions in 24 instances, 3 dilutions in 4 instances and 5 dilutions in 1 instance. Six calves, a few days to 2 months old, had titers of 1:32. Titers disappeared within 6 months. (Iowa-NADL) (ADP al-35)

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AREA NO. 2 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF SWINE

Problem. Profitable swine production depends largely on the ability to control diseases. Swine diseases cause losses estimated at more than $200 million annually. In order to control and eventually eradicate these diseases, a thorough knowledge of causes, diagnostic procedures, preventive procedures, and treatments is required. Although a great deal of excellent research has been and is being accomplished, a vast amount of research is still required to obtain this knowledge. At present, the causes of several important swine diseases are unknown or incompletely understood. Extensive fundamental research on swine diseases is essential to the welfare of the swine industry.

USDA AND COOPERATIVE PROGRAM

The Department has a long history of swine disease research. For example, research on hog cholera was initiated in 1884. Research on this and other important swine diseases is a continuing long-term program. Modern research techniques in the areas of biochemistry, biophysics, pathology, microbiology, pharmacology, physiology, and immunology, are being applied to swine disease problems. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 23.3 professional man years. This effort is divided among sub-headings as follows:

Hog Cholera 9.1 at the National Animal Disease Laboratory, Ames, Iowa, the Florida Hog Cholera Research Station, Live Oak, Florida, under a cooperative agreement with the University of Illinois, and under a contract with the University of Nebraska.

Atrophic Rhinitis 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Transmissible Gastroenteritis 3.6 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with Purdue University and the University of California.

Erysipelas 3.6 at the National Animal Disease Laboratory, Ames, Iowa, and in connection with a PL 480 grant to the Institute for Veterinary Research, Pulawy, Poland.

Brucellosis 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

PROGRAM OF STATE EXPERIMENT STATIONS

Swine disease research at the State stations is being conducted on nearly all of the major disease entities of swine present in this country and on a number of other problems newly encountered or of growing importance.

Ten States are cooperating with the Department in a regional attack upon swine enteritis. These studies include identification and typing of bacteria found in outbreaks of this disease in an effort to trace the cause to specific bacterial types. The role which viruses play in causing intestinal disease outbreaks is under study - this includes joint efforts to develop diagnostic agents and immunologic procedures for transmissible gastroenteritis. Several States are using germ-free swine to determine the disease producing capabilities of single bacterial or viral species. Causes of disease outbreaks in Specific Pathogen Free swine herds are being studied to perfect this disease control procedure.

Other work is in progress to determine the causes of atrophic rhinitis and to develop practical means of prevention. More rapid and practical tests for diagnosing hog cholera are being developed and improved methods of immunization are being sought.

Increased emphasis is being placed on determining the cause and methods of preventing stomach ulcers in swine. Basic investigations are under way to evaluate the role of nutrition in swine disease and the mechanism of immunity in specific infections. The role of sensitization phenomena in causing the arthritic form of erysipelas is being studied.

The total State scientific effort devoted to swine disease research is 18.4 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Hog Cholera

Research at the National Animal Disease Laboratory, Ames, Iowa, was conducted in the following phases:

1. Antibody Reactions with Hog Cholera Virus. A soluble precipitating antigen (HCA) has been obtained from tissue cultured swine kidney cells infected with hog cholera virus. The antigen forms a single precipitin line in agar double diffusion with homologous antiserum. The specificity of the reaction was corroborated by the facts that hog cholera-immune sera did not react with control antigen, and nonimmune sera did not react with either the viral or control antigens. The antigen was readily separated from infective virus by DEAE-chromatography and by high-speed centrifugation. Following certain conditions of exposure to viable hog cholera virus, swine develop precipitating antibodies for HCA.

2. Heat Inactivation of Hog Cholera Virus. The hog cholera eradication program has made it necessary to obtain the answers to many unanswered questions. One of these questions is what temperature is required to kill the virus in pork and pork products. A total of 49 tests was made and 85 head of pigs were used to establish the effects on hog cholera virus blood, of heating at different temperatures for various lengths of time. Hog cholera virus in defibrinated blood was inactivated when heated at 69°C for 30 minutes, but was not inactivated when heated at 60, 62, 64, 66, and 68°C for 30 minutes. When the heating time was increased to 45 and 60 minutes, the virus was inactivated at 68 and 66°C, respectively.

When the preheating time was increased from 3 to 120 minutes and the heating time kept at 30 minutes, the virus was inactivated at 68°C. It was not inactivated at 66 C when the preheating time was increased to 140 minutes. Blood containing virus, when heated to 97 C, or boiling and then immediately cooled, was not inactivated. Virus in blood diluted to 80 percent with physiological saline (0.85 percent NaCl) was not inactivated at 68 C for 30 minutes. Virus in serum was attenuated when heated to 68 C for 30 minutes and inactivated when heated to boiling momentarily at 97°C.

3. A Study of Farm Swine Herds Vaccinated with Crystal Violet Glycerol Hog Cholera Vaccine. A five-year study was made of the same farm swine herds vaccinated each year to determine a) the ability of pigs vaccinated at the farm to withstand exposure to virulent hog cholera virus 1, 3, and 6 months after vaccination, b) the factors responsible for the inability of vaccinated pigs to withstand exposure to virulent hog cholera virus, c) the efficacy of a yearly vaccination with crystal violet glycerol vaccine in herds on the same farms for the prevention of naturally occurring hog cholera, and d) the efficacy of double vaccination of pigs that did not develop satisfactory immunity against exposure to virulent hog cholera virus after a single vaccination.

Crystal violet glycerol (CVG) vaccine was used to vaccinate 67,058 farm pigs from 1956 to 1960. Two test pigs from each herd were taken to the Federal Hog Cholera Res. Station at Live Oak, Fla., 1,3 and 6 months after vaccination and exposed to 1,000,000 MLD of virulent hog cholera (HC) virus under controlled conditions. A total of 2,931 pigs or 4.37 percent of all pigs vaccinated were exposed to HC virus. The percent survival for the 1, 3, and 6 month vaccinates was 86.33, 87.80, and 90.78, respectively.

Of 1,236 pigs tested 1 month after vaccination, 60.18% had good protection, 15.53% had fair protection, 10.51% had poor protection, and 13.67% had no protection.

Of 1,229 pigs tested 3 months after vaccination, 49.71% had good protection, 19.60% had fair protection, 18.22% had poor protection, and 12.20% had no protection.

Of 466 pigs tested 6 months after vaccination, 44.63% had good protection, 23.39% had fair protection, 22.74% had poor protection, and 9.22% had no protection.

The percent protection of the 1, 3, and 6 month vaccinates, based on their reaction to HC virus, was 71.91, 67.81, and 67.27, respectively.

Subclinical infection with Pasteurella spp. or Salmonella cholera suis is believed to have adversely affected the ability of pigs to withstand exposure to HC virus. An unknown agent responsible for a bloody diarrhea also interfered with the ability of pigs to withstand exposure to HC virus.

Hog cholera did not occur in any farm herd vaccinated with CVG vaccine. Hog cholera was known to have occurred, however, on many farms in the same area and on four farms adjacent to farms where CVG vaccinated pigs were being raised. Double vaccination with CVG vaccine induced over 95% protection in pigs in four herds where a single vaccination induced less than 30% protection. (Ames, Iowa - NADL) (ADP a2-17(c)

4. Pilot hog cholera eradication field studies to evaluate hog cholera vaccines. The evaluation of experimental field trial hog cholera eradication in Suwannee County, Florida, was begun in April, 1957, and was terminated December 31, 1962. The study was designed to measure the potency, safety, and shelf life of three types of commercial modified live-virus vaccines (lapine, porcine, and tissue culture origin), administered with a minimum of 15 ml. of anti-hog cholera serum. Records were kept on all vaccinated and unvaccinated swine herds.

A summary of the studies and final report, including conclusions, is as follows:

(1) Average annual swine vaccination coverage in the pilot hog cholera eradication area for the period was 73.1 percent. (2) Post-vaccination challenge of 4,842 pigs showed that 87.8% were adequately protected while 91.2% survived. (3) The farm-to-farm variation in the ability of pigs to develop an adequate immunity over the 69-month test period showed an average difference of 25.7 percent. (4) Twenty ml. or larger doses of anti-hog cholera hyperimmune serum, administered simultaneously with modified live-virus vaccines of all types, resulted in lower percentages of adequately protected pigs than smaller serum doses. (5) Stress factors recorded in 206 herds at time of vaccination had no significant effect on the development of immunity. (6) The most significant factor found to have an adverse effect on the percentage of adequately protected pigs was vaccine age at the time of vaccine administration. (7) Fifty-eight cases of hog cholera were confirmed during the period, of which 63.8% occurred in farm-raised swine. (8) More than one half of the feeder pigs (52.8%), delivered for sale to the public market, were not vaccinated.

After December 31, 1962, the field trial study was changed to measure the spreading characteristics of modified live-virus vaccines administered with and without hog cholera antiserum for the six basic patents or patents-pending covered by commercial production. In this program 945 pigs in 18 herds were vaccinated with 4 serials of modified live-virus vaccine manufactured under 2 patents with 10 ml. of antiserum per pig, and 530 pigs in 15 herds were vaccinated without serum. Non-vaccinated contact controls were left in each herd. Thirty days after vaccination, vaccinated pigs and an equal number of non-vaccinated contact control pigs were purchased for challenge. Six months after vaccination, or when the pigs were ready for market, whichever occurred first, another set of vaccinated pigs and non-vaccinated contact control pigs were purchased for challenge. At the present time 192 pigs from 49 herds have been challenged. The results of these challenges are incomplete.

Vaccination of swine with killed-virus vaccines was continued under the program for Lowndes County, Georgia, until March 10, 1964. Vaccinations are now being done privately, using killed vaccines. Challenge work of Lowndes County vaccinates continues to indicate two doses of killed-virus vaccines, 30 days apart, produce a higher level of immunity than 1 dose, even with the use of antiserum at the first vaccination. (Live Oak, Florida) (ADP a2-13)

5. Diagnosis of hog cholera. In research being carried out under a cooperative agreement at the University of Illinois, Urbana, cytopathogenic effects were observed in tissue cultures inoculated with hog cholera virus and incubated under increased oxygen tension. One difficulty in working with this virus heretofore was that it produced no visible effect in tissue cultures. This procedure holds promise as a diagnostic test for hog cholera. In other studies at the University, intradermal injection of attenuated hog cholera virus resulted in skin reactions in 15 percent of the swine that had been exposed to the virus 5 or more days previously.

Although the intradermal skin test appeared to be specific, the low reactor rate makes it of limited value as a diagnostic procedure. (Illinois)

In research conducted under contract at the University of Nebraska, Lincoln, a highly promising test for the diagnosis of hog cholera using a fluorescent antibody staining technique has been developed. The test is accomplished directly on fresh tissues such as tonsil, lymph nodes, salivary gland, and kidney. In experimental cases, hog cholera virus could be detected in the tonsil as early as 72 hours after exposure. (Nebraska) (ADP a2-17(C)

B. Atrophic Rhinitis

Research on atrophic rhinitis at the National Animal Disease Station, Ames, was temporarily suspended during the year. During this coming year efforts will be directed toward developing a swine herd free of turbinate anomalies and other diseases, to be used specifically on this project. (Ames, Iowa) (ADP a2-8(Rev.)

C. Transmissible Gastroenteritis (TGE)

In research studies at the National Animal Disease Laboratory, Ames, two isolates of virus from transmissible gastroenteritis of swine were adapted to grow on primary swine kidney cells. Purification and more characterization of the virus is now made possible. Previously, studies with this virus were limited to those findings which could be made in young specific-pathogen-free pigs which were relatively expensive and in limited supply. The tissue culture adapted viruses make possible a many-fold increase in the number of experiments that can now be accomplished. All previous attempts to grow transmissible gastroenteritis virus in tissue culture at this laboratory had been unsuccessful. Animal inoculation, virus interference, serum neutralization, and fluorescent antibody staining were techniques used to identify the isolates growing in the cell cultures. All confirmed the observation. (Ames, Iowa-NADL)

At the University of California, research is being conducted, through a cooperative agreement, on the enteroviruses of swine and their interrelationship. In California, a virus isolated from pigs with diarrhea was shown by neutralization tests to be serologically related to the Teschen group. The histologic changes observed in the central nervous system were indistinguishable from the polioencephalomyelitis noticed in pigs with Teschen disease. (Davis, California)

At Purdue University, Lafayette, Indiana, through a cooperative agreement, research is being directed toward elucidating the mechanisms by which transmissible gastroenteritis (TGE) virus causes diarrheal disease in young pigs and the means by which passive immunity to TGE is conferred from sows to their pigs. The site of replication of TGE virus in pigs is being determined by infecting surgically-isolated segments of the gastrointestinal

tract in anesthetized pigs. It appears that passive immunity to TGE is a result of neutralization of virus within the lumen of the alimentary tract by ingested antibody rather than by action of circulating antibody. (Indiana) (ADP a2-10(Rev.)

D. Swine Erysipelas

There has been relatively little information pertaining to the underlying physiological changes in pigs during swine erysipelas infection. At the National Animal Disease Laboratory, Ames, Iowa, a study has been completed in which hematological, physiological and bacteriological parameters were measured in 13 pigs before and after infection with Erysipelothrix rhusiopathiae. Considerable effort was devoted to development of recording and sampling methods which minimized disturbance of the animals. Based on severity of clinical response, the animals were placed into three groups following infection: 1, acute with death; 2, acute without death; and 3, subacute. The most striking and consistent hematological change was leukocytosis followed by leukopenia. An initial shift to the left in the blood count was followed by an increase in lymphocytes and later by an increase in immature neutrophils. The cell sedimentation rates in groups 1 and 2 were significantly increased while blood pH values in these groups decreased. Groups 1 and 2 also had significant decreases in serum albumin and increases in a-globulin. The reduction in blood glucose paralleled the severity of infection. Blood creatinine was increased significantly in group 1, while blood urea nitrogen increased significantly in all groups. Serum glutamic oxalacetic transaminase increased 5-fold in groups 1 and 2. Blood pressures were consistently lower in all groups following infection, and dropped continuously until death in group 1. Heart rates of animals in groups 1 and 2 were first decreased, then increased, following infection. Maximum increases in body temperature occurred between 60 and 84 hours after infection. Erysipelothrix rhusiopathiae was recovered from all hemocultures from group 1, but was recovered less frequently from hemocultures from groups 2 and 3. The application of more recently available electronic instruments and techniques, coupled with concurrent biochemical and bacteriological tests during the course of erysipelas infection, furnished the means for this physiopathological study.

During the year a cooperative agreement was concluded with Seton Hall College of Medicine (Department of Biochemistry), Jersey City, New Jersey. During this study the organism which causes swine erysipelas, Erysipelothrix rhusiopathiae, was shown to possess five serologically active antigens when assayed by the agar double-diffusion technique. (New Jersey)

An investigation, carried out under a PL 480 grant to the Institute for Veterinary Research, Pulawy, Poland, is aimed at improving diagnostic and immunizing procedures for swine erysipelas. (E21-ADP-8) (ADP a2-15)

E. Swine Brucellosis

Work on swine brucellosis at the National Animal Disease Laboratory, Ames, was conducted on the serology, bacteriology, and histopathology of this disease. This research has not, as yet, progressed to the reporting stage.
(Ames, Iowa) (ADP a2-16)

F. Abscesses in Swine

Research on abscesses in swine was initiated at the National Animal Disease Laboratory, Ames, but has not progressed to the reporting stage.
(ADP a2-19)

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AREA NO. 3 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF SHEEP AND GOATS

Problem. There are at least 18 infectious diseases of sheep and goats in the United States that cause an estimated annual loss of 15 million dollars. Non-infectious diseases are estimated to cause an additional 3 million dollar loss annually. The cause of some of these diseases is known; others have more than one causative agent contributing to produce the effects seen in field cases. Environmental, genetic, and unknown factors appear to play a part in some diseases. The natural reservoirs of the known infectious agents have not been fully determined. Fundamental information on methods of transmission and means of prevention are needed for many of these diseases. Vaccines and other immunizing products are available for some diseases of sheep but not for others. Some of these products might be improved. Prevention, control, or eradication of disease is necessary for economic and efficient sheep and goat raising. Due to lack of accurate, rapid diagnostic techniques, infectious diseases often get a substantial start in a band or flock before they are recognized, partly because they are easily confused with non-infectious diseases.

USDA and COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of sheep and goats. Research is being conducted on the diseases at the following designated locations.

The Federal scientific effort devoted to research in this area totals 9.9 professional man-years. This effort is applied as follows:

Bluetongue, 4.0 at the Denver Animal Research Laboratory, Denver, Colorado.

Contagious Ecthyma, 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Foot Rot, 2.0 at the National Animal Disease Laboratory, Ames, Iowa

Vibriosis, 0.6 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Colorado, Montana, and Utah Agricultural Experiment Stations.

Scrapie, 0.2 at the Agricultural Research Council Field Station, Compton, Berkshire, England, and the Moredun Institute, Edinburgh, Scotland, through two grants of PL 480 funds, equivalent to $300,165. The work is coordinated through the European Mission for Research on Animal Diseases, Amsterdam, Holland.

Viral Ulcerative Dermatosis, 0.1 through a cooperative agreement with the Colorado Agricultural Experiment Station.

Paratuberculosis or Johne's Disease, 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

PROGRAM OF STATE EXPERIMENT STATIONS

Considerable attention is being given to many of the diseases of sheep and goats in order to reduce the cost of production thereby encouraging consumption of meat, wool and by-products of the industry.

At the present time, several States in the West (Regional Research Project W-27, Vibriosis in Sheep) are cooperating to develop methods for the prevention and control of vibriosis, one of the major disease problems of the sheep producer. Information is being sought on how the disease is transmitted and on the source of infection. Preliminary reports indicate that preventive vaccines offer promise as an aid in the control and eventual eradication.

Considerable emphasis is being placed on the development of reliable tests that can be used to identify outbreaks of bluetongue and the detection of carrier animals. Vaccines for the prevention of bluetongue are being evaluated by workers at several stations. Vectors, in addition to those already known, are being sought in order to improve present control measures.

Many States are giving attention to the prevention and control of white muscle disease (myodegeneration) in sheep and the relationship of the condition to similar problems in other animals, including man.

The influence of nutrition on the physical and chemical properties of urine is being studied to determine the cause of urinary calculi. Methods for prevention and treatment are being evaluated.

Conditions known as epididymitis and ulcerative dermatosis have become economic problems in some areas and several States are devoting considerable effort to determine means of control.

Other sheep and goat diseases being investigated by workers in various States are pneumonia, listeriosis, foot rot, ovine virus abortion, encephomalacia, etc.

The total State scientific effort devoted to diseases of sheep and goats is 20.2 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Bluetongue

1. Bluetongue virus isolations were made at the Animal Disease Research Laboratory at Denver, Colorado, from sheep blood samples representing suspected bluetongue outbreaks in 12 bands of sheep from 7 States, and from cattle blood samples from 6 herds from 3 States.

2. Detection of bluetongue virus in infective cell culture utilizing fluorescent antibody and electron microscopy. Bluetongue virus has been visualized in lamb primary kidney cell cultures and in serially passed McCoy Synovial cell cultures. The infective cycle has been determined utilizing goat bluetongue antiserum specifically conjugated with fluorescent isothiocyanate. The first indication of bluetongue infection occurred at 20-30 hours after the cells were inoculated, the cycle was complete within 40-60 hours of inoculation. The first sign noted was around the periphery of the cell where a thin band of apple green fluorescence was noted. This specific fluorescence moved toward the nucleus becoming diffuse in the cytoplasm and became concentrated around the nucleus at approximately 30-40 hours after culture inoculation. The first stage occurred when the fluorescence moved away from the nucleus again toward the periphery. Shortly after this phase of the cycle the cells underwent lysis and fell off the glass substinate.

Cytoplasmic inclusion bodies were noted in the cultures as early as 25 hours, however, at this time there was none-to-weak fluorescence. After 30 hours incubation the inclusion bodies absorbed the specific conjugate with increasing brilliance until the cells underwent lysis. Cultures were studied 36 hours after inoculation with bluetongue virus utilizing the ultramicrotone. Mature and immature virus was noted in the areas associated with specific fluorescence. Electron microscopic studies of inclusion bodies showed virus particles within the structure. Virus measured directly on the photomicrograph was found to be elliptical and 100 x 80 millimicrons in size. The virus consists of a ribonucleic acid (RNA) core surrounded by a clear capsule.

3. The clinical and immunogenic response of sheep to oral and intradermal inoculation of bluetongue (BT) virus. All principal sheep became infected subsequent to intradermal inoculations of 1 ml. of a dilute (10-3) bluetongue virus 3 times per week for 26 weeks. The incubation period was prolonged. One contact control sheep developed bluetongue. Two of 7 sheep, on a like test, that received 1 ml. of dilute (10-5) bluetongue virus responded. One of the 2 sheep reacted to homologous challenge, whereas neither had significant serum neutralization indexes. Five of 7 sheep that received oral inoculations of 0.25 ml. of bluetongue virus blood in an anticoagulant preservative solution (OCG) 3 times per week for 26 weeks showed clinical reaction. Only one had a significant serum neutralization index and resisted homologous challenge.

4. The viremia of bluetongue infected sheep. Bluetongue virus was successfully titered, for the first time, directly from sheep blood in embryonating chicken eggs.

Four sheep were infected with BT-262 virus isolated during the first week of July, August, September, October, and December, for a total of 20 principal sheep. Two additional sheep served as non-infected temperature control sheep for each separate month's experiment. Blood samples were collected in OCG for 21 consecutive days from the principal sheep. The average peak BT virus activity, measured by the total number of BT virus chicken embryo mortalities, occurred on day-after-inoculation (DAI) 7. The bulk of the virus activity occurred on DAI 4 through 10 with the intermittent detectable virus present as early as DAI 1 and as late as DAI 21. The individual sheep virus titers, expressed as the log titer LD_{50} per 1 ml. blood in OCG, ranged from zero to 4.0. The zero titering blood had detectable virus present on DAI 6 through 10, and again on DAI 17. Blood virus collected in August gave the highest and most consistent titers. During 21 consecutive days of virus assay, the sheep with the best viremia had the highest virus titers.

5. Enhancement of sheep's response to oral doses of bluetongue virus. Forty eight sheep of similar age, weight, and sex were utilized to study the influence of orally administered bluetongue virus on the subsequent BT clinical response when the sheep was given an intradermal injection of the homologous virus. The sheep were divided into 5 separate groups. Group I had 14 principal and 8 virus control sheep. The principal sheep were given 2 ml. of blood virus in OCG daily for 5 days and then inoculated intradermally with the same virus on the day after oral administration (DAOA) 29. The virus control sheep were treated identically and representative of all control sheep with the exception that the oral inoculum was normal blood in OCG. Group IIA had 10 principal and 3 control sheep. In this group the oral inoculum was 4 ml. of blood virus in OCG daily for 10 days and the sheep were challenged on DAOA 15. Group IIB and Group IIC each contained 3 principal and 1 control sheep and they received the same oral inoculum as the Group IIA sheep. However, they were challenged on DAOA 22 and 29 respectively. Group III had 3 principal and 2 control sheep in which each of the principal sheep were given a single oral blood virus inoculum of 10, 20, or 30 ml. One virus control sheep was given a single oral inoculum of 10 ml. and the other 30 ml. of normal blood in OCG. These sheep were challenged on DAOA 11. The most optimal enhancement of the clinical response of the sheep occurred in the Group IIA sheep. The enhancement response was reflected by more severe and prolonged mouth lesions, a marked leukocytosis following a longer duration of the leukopenia, and a slight increase in the daily average body temperatures.

6. Thermostability. The thermostability of bluetongue virus at various storage conditions and temperatures of inactivation was studied at pH 7.0. The virus was markedly thermostable, withstanding 3 years storage at room temperature. Thermal inactivation curves suggested first-order kinetics,

with two components at 37, 46, and 56° C, but only one at -70°C. The two-component curves were most likely due to a phenotypically determined heterogeneity of the virus population with respect to thermostability. While inactivation at high temperatures (46 - 56°C) was associated with marked changes in enthalpy and entropy, compatible with protein inactivation, the thermodynamic data obtained at a lower temperature range (36 - 46°C) suggested ribonucleic acid inactivation. Approximate energy of activation values below and above 37°C were 7.5 and 50 kcal. $mole^{-1}$ respectively.

7. Effect of different "Contact Conditions" on the bluetongue virus-antibody reaction and on the validity of the "Percentage Law". The effect of various times and temperatures of virus-antibody contact (contact conditions) on the bluetongue virus-antibody reaction was studied. Linear regressions of neutralized virus on antibody titers were compared in three different in vivo neutralization tests. In all three tests antibody titers were highly dependent on the virus test dose used, that is, the slopes of the regression plots were flat. In the two in ovo neutralization tests slow virus multiplication probably caused the flat slopes.

In conventional neutralization tests with limited contact conditions, the "percentage law" was invalid at low virus doses. With more favorable contact conditions the range of virus doses over which the regressions were linear and significant was extended gradually. Thus, the "percentage law" became valid for all virus doses. The invalidity of the law at low virus doses in conventional tests was most likely due to the inability of weak virus-serum mixtures to react to equilibrium in such tests. Changes in contact conditions did not significantly affect the slopes of the neutralization plots when these plots were based only on data in agreement with the "percentage law".

No reversibility of the bluetongue virus-antibody reaction was demonstrable by dilution of reaction mixtures at neutral pH. Reaction mixtures were held at both conventional and extended contact conditions.

Antibody titers were increased up to a hundredfold when extended contact conditions were compared with conventional methods. (Denver, Colorado)
(ADP a3-5)

B. Vibriosis in Sheep

In work under a cooperative agreement with the Colorado State University at Fort Collins, a study was made to determine the duration of immunity against ovine vibriosis which began November 1963 by vaccinating a group of yearling ewes prior to breeding. Vaccination was accomplished by giving a single 5 cc. subcutaneous injection of formalin-killed Vibrio fetus serotype I and serotype V organisms, mineral oil adjuvant, bivalent bacterin. An additional group of unvaccinated yearling ewes were maintained as controls. Ewes were randomized into separate lots and pens. At increasing yearly

intervals since vaccination the immunity of vaccinated and unvaccinated controls will be challenged at 2, 3, 4, 5, and 6 years of age. Immunity of 2-year-old ewes was challenged during advanced gestation, April 1964, with the combined V. fetus type I and type V (1:1 ratio) culture challenge. Twenty-three ewes, unvaccinated immunity challenge controls for the combined V. fetus type I and V organisms, had 9 vibrionic abortions. A single abortion occurred in 23 ewes vaccinated with the combined serotype I and V organisms when their immunity was challenged with the combined I and V V. fetus serotypes. No abortions occurred in the unvaccinated, unchallenged ewes which served as negative controls. (Fort Collins, Colorado)

In cooperation with the Montana Veterinary Research Laboratory of the Montana Agricultural Experiment Station at Bozeman, work has continued on vibriosis. An outbreak of vibriosis due to an unusual serotype was observed in a flock of ewes at this laboratory which had been vaccinated for vibriosis.

Antigens have been made and immunization of rabbits started. Serums will be adsorbed with cells in attempts to obtain pure serums representing the various "H" antigen factors. The serums will then be used to study the antigenic patterns of isolants, particularly those of unusual serotypes. Some improvement has been made in procedures for isolating vibrios from ovine feces. V. fetus has been recovered from feces for up to 25 days after rumen inoculation. A Vibrio which resembles V. fetus has been recovered from bovine feces. Two cultures of V. fetus, isolated from placentas during normal lambing of a farm flock in 1963, proved to be pathogenic for pregnant ewes when administered by rumen injection. It appears that the infection can be maintained from one season to the next in a flock although the lambing performance is normal. It is possible that the infection is maintained in carriers. (Bozeman, Montana)

In cooperation with the Agricultural Experiment Station at Logan, Utah, the replacement yearling ewes of 2 herds with a total of about 2,000 ewes each, were vaccinated for the 4th year with Vib-vac (Baldwin Laboratories, Omaha, Nebraska). No V. fetus organisms could be isolated from 48 abortions of the first and from 25 abortions of the second herd.

The duration of immunity of ewes vaccinated ½, 1½, 2½, and 3½ years ago was determined. Infection of the placenta, or the fetus, or both, with V. fetus was taken as failure. No V. fetus organisms were isolated from 15 ewes serving as normal controls, 15 of 16 ewes had infected uterine contents in the infected control group, while the rate of uterine infection was 8/16 ½-year after vaccination, 3/16 in the 1½-year group, 2/16 in the 2½-year group, and 4/16 in the 3½-year group. The u test proved highly significant protection in all 4 vaccinated groups.

Studies on the dynamics of the V. fetus infection were continued. The pooled logarithmic rate of clearance for a coccoid V. fetus strain was found to be -0.0128 which was significantly different from 3 other V. fetus strains with typical, comma-shaped cellular morphology. One of the latter was a V. bubulus strain being cleared from the ewes blood at a rate of -0.090, which did not differ from the clearance rate -0.0974 of a V. fetus strain of bovine origin. The clearance rate of 0.0576 of a typical ovine V. fetus strain, however, differed significantly from the latter 2 strains. The coccoid V. fetus strain, which remained for a long period in the blood stream, was lethal to all intravenously exposed ewes 5½ to 12 hours after inoculation. The animals developed a shock-like syndrome and died.
(Logan, Utah) (ADP a3-1(Rev.)

C. Scrapie

Investigations of scrapie in sheep and goats at the Agricultural Research Council Field Station, Compton, Berkshire, England, and at the Moredun Institute, Edinburgh, Scotland, have continued under the terms of the agreement.

Scrapie was first diagnosed in the United States several years ago. It is, however, not considered to be firmly established and efforts are continuing to eradicate it. Research has been conducted on this disease in Scotland and Great Britain for several years. The U. S. Department of Agriculture is supporting this research through PL 480 grants. In recent years, it has been determined that the disease is probably caused by a transmissible agent. The agent has, however, not been isolated nor characterized in detail. There is also increasing evidence that a certain genetic constitution is existent which determines susceptibility. Additional information is required about the disease before eradication procedures may be improved. Significant progress has been made in that the disease has been transmitted to mice and in this species the incubation period is 4 months, contrasting to the incubation period in sheep of 4 to 36 months. The disease is being transmitted serially in mice and efforts are continuing to adapt the transmissible agent to other species of animals. Efforts are also being made to isolate the transmissible agent and adapt it to tissue cultures. One of the things most needed and required before significant progress may be made on scrapie research is a rapid assay technique. Adapting the transmissible agent to tissue cultures appears to offer the most promise. A good biochemical approach is also being made to isolate the causative agent of scrapie from tissues from affected sheep, goats, and mice. In addition to the biochemical work under way, physicists are studying tissues from diseased animals using electronmicroscopy techniques in an effort to pin point the specific areas where the tissues are affected. The interest in research on scrapie has increased in the past two years and it is quite likely that due to this increased interest, significant progress will be made in developing a better understanding of the disease. (ADP a3-3)

D. Viral Ulcerative Dermatosis

In cooperation with the Colorado Agricultural Experiment Station, the disease was encountered in ewes and rams in the field. Materials collected were used to reproduce the disease in experimental sheep, and from these experimental sheep a secondary transmission was made. Pooled exudates from these were sent to the Wyoming University for virus propagation in tissue culture. A cytopathogenic agent was isolated. (Fort Collins, Colorado) (ADP a3-4)

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AREA No. 4 - DISEASES AND PARASITES OF HORSES

Problem. Currently there are about 3,250,000 horses in the United States, valued at approximately $860 million. About one million of these are draft animals. Considerable numbers of horses and mules are still required for work on cattle ranches and as pack animals. The annual overall value of the horse industry has been estimated at about $1.5 billion. The horse may be an important link in epizootiology of animal diseases in general. Equine piroplasmosis is an acute, subacute, or chronic tick-borne disease of horses that was first recognized in this country in Florida in 1961. It is characterized by high fever, progressive anemia, jaundice, edema, extreme weakness and depression. Fatalities range from 5 to 50 percent of infected animals. This disease, now apparently well established in Florida, has extended into Georgia and poses a serious threat to the entire equine population in the southern United States. The disease is clinically indistinguishable from equine infectious anemia. Horses which have clinically recovered from piroplasmosis usually remain carriers of the disease and are a potential source of infection. African horsesickness, a highly fatal disease of equines, that was confined to Africa until recently, is presently causing serious losses in the Middle East and parts of Asia.

USDA and COOPERATIVE PROGRAM

The Department has recently started a continuous long-term program involving biochemists, pathologists, protozoologists, and veterinarians to work on equine piroplasmosis. In order to be prepared in the event of introduction of African horsesickness into the United States, the Plum Island Animal Disease Laboratory has obtained African horsesickness viruses and antiserums from South Africa. These materials are thus directly available for diagnostic and vaccine studies should the need arise.

The Federal scientific effort devoted to research in this area is 5.5 professional man-years. This effort is divided among sub-headings as follows:

Serological diagnosis, transmission, and control of equine piroplasmosis 3.2 at the Beltsville Parasitological Laboratory, Beltsville, Maryland (In cooperation with the Entomology Research Division).

Chemotherapeutic methods of prevention, treatment, and eradication of piroplasmosis in horses 1.1 under contract with the University of Florida, Gainesville.

Development of antigenic material for a diagnostic test for equine piroplasmosis 1.2 under contract with the University of Kentucky, Lexington.

PL 480 funds have been made available in Turkey for research on Gastrophilus pseudo-hemorrhoidalis (equine parasite) in Turkey; its distribution, life cycle, economic importance, treatment and control.

PROGRAM OF STATE EXPERIMENT STATIONS

While all the colleges of veterinary medicine are active in the treatment and control of equine conditions only a few States are engaged in basic research designed to provide useful fundamental knowledge for the control and eradication of diseases of horses.

Studies are being conducted on the pathology of bones, tendons, muscles and joints involved in lameness in order to develop a more scientific basis for the control and treatment of such conditions. Basic studies of equine lameness complement and extend knowledge concerning athletic injuries in man and bone and joint disease in all animals.

Diseases caused by parasites, fungi, viruses, bacteria and other microorganisms are being studied. Emphasis is being placed on the pathogenesis, transmission, treatment and control. Preventive vaccines are under development and are being tested under laboratory and field conditions to measure their effectiveness in controlling such conditions as arteritis and rhinopneumonitis. More accurate tests for laboratory identification of diseases such as equine infectious anemia and equine piroplasmosis are receiving increased attention.

Parasite-free foals are being used to study the migration, immunity and chemotherapy of various experimentally-induced parasite infections. The evaluation of antiparasitic drugs for activity and safety is being continued in many States.

The total State scientific effort devoted to equine disease research is 7.1 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Equine Piroplasmosis

The Beltsville Parasitological Laboratory (BPL) research workers report that the recognition of equine piroplasmosis in the southeastern United States has opened the question of the biologic relationship between the disease and its vectors in the Western Hemisphere. The tropical horse tick (Dermacentor nitens) is commonly found on horses in many tropical regions of this hemisphere and has been considered the probable vector of equine piroplasmosis although never proven to be. Engorged adult female ticks were collected in Florida from groups of horses in which the piroplasma organism had been found. The ticks were held in the Laboratory until they laid eggs. When the eggs hatched, the larvae were placed in the ears of two adult test horses. Both horses developed equine piroplasmosis (Babesia caballi),

indicating that the female ticks had transmitted the disease organisms to their progeny. (Beltsville, Maryland)

Research studies were conducted at the Agricultural Experiment Station, University of Florida, under contract with the USDA. The following results were obtained on the treatment of horses for equine piroplasmosis:

A. Total number of animals treated.
B. Number of animals treated and determined to be no longer carriers of EP* by use of available means.
C. Number of animals treated and are negative for EP by use of available tests completed to date.
D. Number of animals treated and still carriers of EP as determined by available means.
E. Number of animals removed due to drug toxicity, death not related to treatment or EP, and animals treated for EP in the acute phase but died.

	A	B	C	D	E
Phenamidine	5	1	4	0	0
Sodium Cocodylate	1	0	0	1	0
Acaprin	1	0	0	1	0
Diampron	6	0	0	1	5
Berenil	6	1	3	1	1
Tetracycline	2	0	0	2	0
Total	21	2	7	6	6

*EP - Equine piroplasmosis (Florida) (ADP b6-13(C)

The viruses and antisera for African horsesickness are being held available at the Plum Island Animal Disease Laboratory.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Equine Piroplasmosis

Roby, T. O., and Anthony, D. W. 1963. Transmission of equine piroplasmosis by Dermacentor nitens Neumann. J. Amer. Vet. Med. Assn. 142(7):768-769.

Roby, T. O., Anthony, D. W., Thornton, C. W., Jr., and Holbrook, A.A. 1964. The hereditary transmission of Babesia caballi in the tropical horse tick, Dermacentor nitens Neumann. Amer. J. Vet. Res., 25(105): 494-499.

AREA NO. 5 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF POULTRY

Problem. Annual losses from infectious and non-infectious diseases of poultry, exclusive of parasitisms, are estimated to be at least $200 million. Continued and expanded basic and applied research are essential to aid in reducing these losses, which inevitably affect cost to the consumer. Added to the initial losses from mortality, reduced weight gains, poor feed utilization, decreased egg production, and lowered quality, are the final losses occasioned by condemnations at dressing plants. United States turkey growers in particular, are faced with a new problem in that a newly discovered infection with a different strain of Mycoplasma is widespread in flocks throughout the country. Resulting condemnation losses at slaughter are often great. The problem is to keep abreast of changing conditions in the field, which present increasingly complex problems requiring basic information.

USDA AND COOPERATIVE PROGRAM

The Department has a long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of poultry. Research is being conducted on the diseases at the following locations.

The Federal scientific effort devoted to research in this area totals 31.3 professional man-years. This effort is applied as follows:

Ornithosis 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Universities of California and Minnesota, and the Agricultural Experiment Stations of Oregon and Texas.

Salmonellosis 3.0 at the National Animal Disease Laboratory, Ames, Iowa, and the Southeast Poultry Research Laboratory, Athens, Georgia.

Pasteurellosis 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Chronic Respiratory Disease Complex 16.7 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the Agricultural Experiment Stations of Connecticut, Delaware, Georgia, Massachusetts, New York, North Carolina, Texas, Virginia, and Wisconsin, and with the University of Minnesota.

Newcastle Disease 4.2 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the University of Maine and the Wisconsin Agricultural Experiment Station, and under a PL 480 Grant to the Institute for Veterinary Research, Pulawy, Poland.

Leukosis 0.3 under cooperative agreement with the Regional Poultry Research Laboratory, USDA, East Lansing, Michigan.

PROGRAM OF STATE EXPERIMENT STATIONS

The major effort by the State stations in this area is being placed on the respiratory disease complex (Airsacculitis) of poultry. Twenty-seven States are cooperating with the Department in three regional projects (NC-65, NE-5, and S-34) on basic and applied aspects of this disease complex. The Department also contributes significantly to this research through cooperative agreements with a number of States. Considerable emphasis is being placed on Chronic Respiratory Disease (CRD). Efforts are being made to improve diagnostic procedures through studies of biological and serological aspects of the causative agent and closely related organisms. The interaction of other infectious agents in causing CRD are being explored. The role of environment in disease outbreaks is being examined and practical methods of treatment and eradication are being developed.

Basic studies are in progress at a number of locations on the structure and composition of Newcastle disease virus. The immunogenic properties of various strains of this agent also are under evaluation with the object of providing improvements on present vaccines and diagnostic materials. The role of nutrition in relation to this disease is being determined. Infectious bronchitis and laryngotracheitis are additional important diseases of the airsacculitis complex being studied. Research is being concentrated on antigenic and immunologic characteristics of these diseases to provide improved preventive methods. Tissue culture modified viruses are being evaluated as improved vaccines.

A number of States are studying the growing problem of Salmonellosis and are tracing the sources of this infection. The effects of nutrition and intercurrent diseases on resistance to Salmonella are being determined. Improved methods of diagnosis and control are being developed for ornithosis. Methods of transmission and immunization are being sought for avian leukosis. The causes of diseases such as infectious synovitis, dissecting aneurysm and atherosclerosis are being investigated.

The States are alloting 52.9 professional man-years to poultry disease research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Ornithosis

At the National Animal Disease Laboratory, Ames, Iowa, basic research is in progress on this problem. It is directed toward relationships between psittacosis-group agents found in wild birds and those found in domestic birds and mammals; the differences in their specific antigens, inter-species susceptibility, and other factors which will aid in characterization of the

agents and development of specific diagnostic antigens for the agents. (Ames, Iowa)

At the University of California, cooperative studies have shown chickens to be susceptible to ornithosis agents of low virulence. The virus was excreted in the saliva and droppings as early as 3 days following intratracheal inoculation. The virus could be isolated from organs in the absence of signs of infection for at least 5 weeks, the longest period tested.

The quantity and quality of the ornithosis complement-fixation (CF) antibody component which reacts with the bacterial antigen was found to differ among individuals and animal species following exposure to ornithosis virus. The titers obtained with the Herellea and ornithosis antigens were not always parallel. From a limited number of trials a more sensitive bacterial antigen for detection of common CF antibody was obtained from organisms propagated at room temperature than those grown at 37°C.

Although different batches of the ornithosis yolk sac antigens reacted with anti-ornithosis serums, marked variations in CF sensitivity was observed among them for the same anti-ornithosis serums. By the use of an agglutination test with a formalin inactivated bacterial antigen, antibodies produced by the Herellea bacterium can be detected to differentiate this infection from ornithosis. (Davis, California)

Continued cooperative studies at the University of Minnesota indicate that the ornithosis virus remains prevalent in the avian population in Minnesota and some adjacent States. One hundred sixteen flocks from Minnesota, Wisconsin and Iowa were tested. Nineteen flocks (16.4%) gave positive reactions by the direct complement fixation test. Ten flocks (8.6%) gave reactions regarded as suspicious. The remaining flocks (75%) were considered negative.

Avian species other than turkeys were also tested serologically and were considered negative.

Parallel complement fixation tests with a bacterial extract antigen and the ornithosis antigen continued to yield a good correlation in results. It would appear feasible to substitute the bacterial antigen for the virus antigen in the presumptive diagnosis of ornithosis in turkeys on a flock basis. (Minneapolis, Minnesota)

At the Oregon Agricultural Experiment Station, Corvallis, an isolate of the ornithosis agent from a sea gull has been reduced considerably in virulence for turkeys by serial passage through mice (40 passages) and chicken embryos (20 passages). The agent has become more pathogenic for mice and chicken embryos. No evidence of a substantial immunity resulted from injection of the mouse-passaged agent into turkeys. A colony of red mites was established for use in transmission studies of ornithosis.

Twenty percent of a group of turkeys negative to the Mycoplasma S6 agglutination test became positive to the test within 7 days after exposure to a dose of virulent ornithosis agent. They became negative again 21 days after the exposure. (Corvallis, Oregon)

At the Texas Agricultural Experiment Station, College Station, turkeys experimentally inoculated with ornithosis and Pasteurella multocida (fowl cholera organism) were studied with various diagnostic methods. A combination of stains using the basic dyes and fluorescent techniques were found to be useful in diagnosis. This combination of methods makes it possible to arrive at a rapid positive diagnosis when compared to virus isolation techniques which take at least one week and usually longer. Serology used in conjunction with the staining of the organism with fluorescent stains gives rapid positive identification of this organism.

In a study of a natural outbreak of ornithosis in turkeys, it was evident that the method of spread in the turkey flock was by direct contact. The infection spreads rapidly from turkey to turkey. When clinical signs first appear, introduction into the flock is evidently of recent origin and is probably made by birds of one kind or another.

It is evident from serologic studies made on people working in our laboratory that exposure may produce a slight degree of immunity or antibody titer without clinical signs being observed. (College Station, Texas)(ADP a5-20)

B. Chronic Respiratory Disease Complex

At the National Animal Disease Laboratory, Ames, Iowa, the following work was accomplished:

1. Storage of Mycoplasma Strains. Survival of Mycoplasma strains (19 of avian origin, 3 human, 3 canine, and 1 saprophyte) in the lyophilized state and at various storage temperatures was studied. The effect of alternate freezing and thawing was also studied. All strains survived the freeze-drying process and at least 3 or 4 years of storage in the lyophilized state. At -26 C, they survived for at least 10 months, but changes were noted in the colony size and growth rate of cultures stored longer than 10 months. At -65 C, however, there was little loss in viable numbers from 12 months of storage, and no changes in the organisms were apparent. There was considerable variation from strain to strain in resistance to alternate freezing and thawing. Of 16 strains tested, 13 withstood the freezing and thawing better than did the bacterium Escherichia coli. At 5 C storage there was considerable variation in survival between strains. The saprophytic strain, C-15, showed no apparent loss after 9 weeks at 5 C. Most other strains showed rather rapid loss of viable numbers when stored at 5 C. (Ames, Iowa) (ADP a5-21)

At the Southeast Poultry Research Laboratory, Athens, Georgia, the following studies were conducted:

2. Mycoplasma gallisepticum Antigen Production. Sufficient plate antigen was produced during the year to conduct 210,000 tests. In addition to our own research program, the Georgia Poultry Diagnostic Laboratory, Gainesville, was supplied with 150,000 test doses.

3. Hyperimmunization of Chickens and Rabbits with Mycoplasma gallisepticum (S6, 801 and A5969). The use of small doses of concentrated antigen injected at proper intervals into chickens and rabbits produced antisera with high hemagglutination inhibition titers. It was found that the time of the inoculation is more important in the production of antibodies than the total amount of antigen injected.

4. Chronic Respiratory Disease Control Program. In a study involving 143,350 broilers, a net savings of 0.347 cents per pound in favor of Mycoplasma-free chicks was demonstrated. Mycoplasma-free broilers cost 11.98 cents per pound to produce versus 12.327 cents a pound for Mycoplasma-positive broilers. Poorest performance occurred where M. gallisepticum-free and commercial birds were mixed in the same houses.

5. Newcastle disease and Infectious Bronchitis Virus Purification. Examination of field and laboratory strains of Newcastle disease and infectious bronchitis viruses collected for research investigations has shown instances of contamination with PPLO. The possibility is widely recognized that some or all of these strains may also be contaminated with "wild" avian viruses. These contaminants would be unnoticed if they did not cause lesions in chickens or in the chick embryo. They also cause misleading results or interpretation of serological experiments and may account for varying results in different laboratories. The contaminants are being eliminated by filtration and limit dilution techniques. (Athens, Georgia) (ADP a5-17, a5-18, and a5-23)

Cooperative studies at the Connecticut Agricultural Experiment Station, Storrs, were conducted on several phases of the problem, viz:

6. Serology. Production of Mycoplasma gallisepticum antigen for chronic respiratory disease (CRD) testing was continued under AIQ special license number 237. Antigen was shipped to 25 States and 9 foreign countries. Approximately 700,000 doses were produced this past year, a 35% increase over the previous year. A CRD testing program on a flock or partial sample basis was made available to the poultry farmers of Connecticut.

7. Control. a) Immunization of 8-12 week old birds with living pathogenic M. gallisepticum was conducted with the goal of producing progeny free of this Mycoplasma. Records of the performance of vaccinated birds were in good agreement. Vaccinated birds performed as well as non-vaccinated birds in regard to growth rate, hatchability, fertility, egg production, and

mortality. In addition, vaccination of birds in one house which was a problem in 1961-1962 resulted in a 10% increase in egg production in 1963. Embryo transmission of M. gallisepticum from birds vaccinated at a young age appear to be negligible and the feasability of producing M. gallisepticum-free birds by this procedure has been demonstrated in at least one case. b) Killed vaccine: Attempts at vaccination against M. gallisepticum with killed vaccine have been rather unsuccessful. The possibility of vaccinating with PPLO killed by gamma irradiation at a level which does not denature protein was explored. c) Isolation: Using a filtration method (Millipore 0.45 micron filters), successful isolations of M. gallisepticum from the trachea of 7 groups of birds were made in which no isolations were made due to bacterial growth in a similar medium containing thallous acetate and penicillin but not filtered. d) M. gallisepticum antigen: The difficulties of growing M. gallisepticum in good yields for antigen production are well known to all working in this area. To overcome these difficulties the research at this laboratory has been in two directions - to provide a more optional medium, and to devise methods for more efficient use of the antigen produced by current methods. (Storrs, Conn.)

Cooperative research at the Delaware Experiment Station, Dover, has been concerned primarily with the investigation of the value of various antibiotics (Aureomycin, Terramycin, Erythromycin and Tylosin) for the control of M. gallisepticum infection in experimentally infected broiler chickens. Tylosin was the most effective antibiotic for the control of M. gallisepticum infection under the conditions of these experiments. While this drug did not completely suppress the infection, it was more effective than the other drugs tested. Aureomycin and Terramycin gave some control of the infection while Erythromycin was the least effective drug employed. (Dover, Delaware)

At the Georgia Agricultural Experiment Station, Athens, cooperative studies showed a) broiler flocks on non-medicated feed did as well as those on medicated feed: b) the success or failure of vaccination programs against respiratory virus diseases depends mainly on the degree of latent Mycoplasma infection: c) CRD-free broilers grow heavier and have fewer condemnations than PPLO-infected birds. Birds from the same breeder flock sources have lower weights and higher condemnations when grown in a controlled environmental house even during the winter months. This was probably due to easier spread of infection through the air in a house with forced draft ventilation. (Athens, Georgia)

At the Massachusetts Agricultural Experiment Station, Amherst, cooperative studies and the results were:

8. Transmission. Transmission of CRD through cohabitation of CRD-serologically positive birds and susceptible birds may occur, under certain conditions, only after a prolonged exposure period. Fecal material of CRD serologically positive birds fed to susceptible birds yielded transmission of the disease.

9. Serology and immunity. a) Parental agglutinin studies. Varying degrees of agglutination of M. gallisepticum (S6) antigen may be produced by sera from day-old chicks obtained from positive dams. Individual dams may produce chicks of similar serologic status, but there may also be considerable variation among the chicks within the same hatch as well as between hatches. Tylosin therapy of the serologically positive dam does not appear to alter the serologic reaction of the dam or its progeny. b) Comparison of serologic tests. Serologic tests (rapid-serum-plate, tube agglutination, and hemagglutination-inhibition) on samples collected from known negative birds showed close agreement. This also appears to be true for known strongly positive birds. However, the results of the different testing methods on samples from some positive birds are not in agreement, even though the results of successive tests on the same positive birds appear to be highly reproducible for the respective methods.

10. Response of CRD to medication. Tylosin, given in adequate dosage, may be highly effective in the control of experimental CRD infection. Eradication of CRD from infected breeding stock may be accomplished through sound management and tylosin medication of dams and their progeny.

11. Control and eradication. CRD-free stock can be produced, maintained, and reproduced if adequate sanitation and preventive practices are adopted. The majority of negative premises continue to remain negative on successive years. Significant progress has been made in establishing CRD-free stock and this stock will become available in larger numbers to the industry in various parts of the country. (Amherst, Massachusetts)

At the University of Minnesota, Minneapolis, the following cooperative studies have been conducted:

A whole blood plate antigen is being used as a screening test for the detection of Mycoplasma gallisepticum infection in chickens. Two experiments are being conducted in an effort to produce M. gallisepticum-free chickens. The pathogenicity of 19 avian Mycoplasma serotypes was determined. The two primary pathogens are the A serotype (S_6 strain) and the H serotype (N strain). The S serotype (infectious synovitis strain) is capable of producing synovitis but not airsacculitis. A few of the other serotypes produce a local lymphofollicular reaction while still other serotypes appear to be non-pathogenic.

Attempts have been made to establish "Mycoplasma free" turkey poults by the use of tylan. A significant reduction in the incidence of airsacculitis in the day-old poult was seen following the dipping of the egg in a solution containing 1000 ppm of tylan. This treatment also resulted in a reduced condemnation from airsacculitis when these birds were marketed at 16-24 weeks of age. The H serotype (N strain) appears to be the primary cause of the airsacculitis seen in the day-old poults.

A satisfactory media has been developed for the growth of the H serotypes of Avian Mycoplasma. This has made it possible to develop a serum plate antigen for the detection of this serotype in Minnesota turkeys. A study of the incidence of this serotype is being planned for the coming year. (ADP a5-17)

Field investigations were conducted on clinical outbreaks of infectious sinusitis on eleven premises during the past year. While two investigations are still pending, none of the outbreaks have been traced back to a Minnesota Mycoplasma gallisepticum tested breeder flock.

Studies were continued on the effect of envionmental conditions on the airsacculitis syndrome in turkeys. Two experiments were conducted in an effort to raise two "Mycoplasma free" fryer roaster turkey flocks. The eggs were dipped in both experiments, in the second experiment the poults received a 5-day treatment of tylan in the drinking water. The airsacculitis seen both at one day of age and at the time of processing was minimal in both flocks. Mycoplasma could be isolated from the egg dipped flock but not from the flock where the eggs were dipped and the poults were treated.

Air samples were taken in the environmental turkey buildings throughout the last two experiments. The count rose to a high level during the first three to five weeks of the experiment and remained high throughout the remaining 10 to 14 weeks. The significance of this data is unknown at the present time. (Minneapolis, Minnesota) (ADP a5-21)

Cooperative studies at the New York State Agricultural Experiment Station, Ithaca, have given the following results:

Mycoplasma gallisepticum was destroyed in eggs when they were dipped in 1500 ppm solution of Spiramycin. Tylosin in concentrations of 1000 ppm has continued to be effective as a dip for infected eggs. Eggs from three infected breeding flocks, dipped in Tylosin solution (eggs at 37C, dip solution 5C, dip time 5 minutes) resulted in 20 clean hatches and one infected hatch. The single failure was due to inadequate warming of the eggs prior to dipping. Of the 31 hatches from the same flocks that were not dipped, 5 were clean and 26 infected. One clean breeding flock (itself derived from dipped eggs) which produced 5 clean hatches from undipped eggs became infected in an unknown manner. In general, dipping of infected eggs in an antibiotic solution promises to be a quick and effective way to produce clean progeny from infected dams.

Immunization of chickens with live cultures to prevent egg transmission of PPLO has two inherent difficulties - a) the delay between immunization and egg production, and b) the risk of inducing complicated CRD when a virulent immunizing culture is used. Non-pathogenic immunizing cultures do not produce solid immunity.

The isolation of M. gallisepticum from tracheal swabs, does not necessarily mean that such birds are spreaders. On the other hand, spread of infection occurred from birds that did not yield PPLO from tracheas. Positive agglutination tests appeared later than culture isolations at the outset of infection. A high incidence of positive serological tests persisted for a much longer time than it was possible to make an equivalent number of PPLO isolations from the trachea. One must consider any bird with a positive agglutination test as a potential spreader. (Ithaca, New York)

Cooperative studies at the North Carolina Agricultural Experiment Station, Raleigh, determined the microscopic changes taking place in the turkey with experimentally produced sinusitis. The reaction in the sinus wall was characterized by an increased blood supply, a progressive invasion by white blood cells, the collection of fluids in the tissues, and the formation of an exudate in the sinus cavity. This study adds to the knowledge which may be used in the diagnosis of natural and experimental sinusitis of turkeys.
(Raleigh, North Carolina)

At the Texas Agricultural Experiment Station, College Station, the following cooperative studies were reported:

Chronic Respiratory Disease Eradication. Preliminary cooperative attempts to bring 20,000 replacement chickens, hatched from M. gallisepticum free breeding stock, into production free of M. gallisepticum failed. Efforts are being continued to develop commercial breeding operations free of M. gallisepticum.

M. gallisepticum Antigens: Cooperative studies have been made with the ADE Division of the USDA to establish standard M. gallisepticum production and testing protocols. USDA experimental lots of antigen have been subjected to extensive evaluation. Lots made with the Adler S-6 strain have not been entirely satisfactory due to granularity and hypersensitivity. Lots of antigen made with the Massachusetts A5969 strain have been quite reliable. Both antigens are more sensitive than antigen made with the Iowa 801 strain. USDA H.A. antigen (Bio-20) fails to detect low titering M. gallisepticum reactors when used according to standard protocol.

Non-Specific Reactions to the M. gallisepticum Plate Test. Preliminary studies demonstrate that turkeys immunized with erysipelas bacterin transitory reactions to the M. gallisepticum plate test. Reactions appeared as early as 7 days post-inoculation and persisted as long as 22 days post-inoculation. The reaction and persistence of reaction levels were higher with USDA A5969 antigen than with TAES 801 antigen.

Turkeys immunized with fowl cholera bacterins apparently do not develop significant transitory reactions to the M. gallisepticum plate test.
(College Station, Texas)

In cooperative studies at the Virginia Agricultural Experiment Station, Blacksburg, the following results were reported:

The ultrastructure of 17 strains (15 species) of Mycoplasmatales was studied with the aid of the electron microscope. The average diameter of mature cells, elementary bodies and ribosomes, and the thickness of the 3 layers of cell membranes was determined. The presence or absence of developing elementary bodies, internal membranes (mesosomes) and vacuoles was determined, and the nature of special features was described.

The morphology and ultrastructure of 2 strains of Mycoplasma gallisepticum was studied with the aid of the electron microscope. Evidence supporting the viewpoint that M. gallisepticum multiplies by the formation of elementary bodies was obtained.

A severe Mycoplasmal salpingitis was produced in turkeys. In chickens, the caseous plug associated with Mycoplasmal salpingitis was found either to be resorbed or to be passed from the vent before the first eggs were laid. In addition, many eggs had little or no mineral deposition on the shell membrane.

"Mycoplasma free" flocks have been maintained at this Station for seven years by appropriate management procedures. (Blacksburg, Virginia)

At the Wisconsin Agricultural Experiment Station, Madison, cooperative studies show that of the variables in the environment of confined turkeys that have been measured, three (temperature, carbon dioxide, and ammonia) are being studied in environmental chambers. Ammonia at the measured level is damaging to the respiratory tract of chickens and turkeys. After prolonged exposure, changes in the tracheal epithelium can be demonstrated histologically. Even after short exposure the birds are markedly more susceptible to Newcastle disease. On the other hand, carbon dioxide at levels that exist in commercial establishments has rendered the birds less susceptible to Newcastle disease.

The only Mycoplasma found in the Charmany study flocks is N strain (H serotype). Primary attention has been given to improved methods for the culture of this strain and to improved methods for its serological detection. The presence of the organism has been followed from the laying flock, to the hatchery, and in the hatched poults from one day of age, by means of a weekly sampling, to the 14-week market age. The condemnation status of each flock is determined by a Federal inspector at an approved plant. The presence of N strain and the extent of respiratory disease in market age birds is being carefully assessed. (Blacksburg, Virginia) (ADP a5-21)

C. Salmonellosis

At the Southeast Poultry Research Laboratory, Athens, Georgia, work on this problem has shown the following results:

1. Salmonella typhimurium Stained Antigen. Further refinements have been made in a stained antigen to detect carriers of Salmonella typhimurium infection in poultry flocks by either the rapid whole-blood or serum plate test. A single culture of S. typhimurium has been selected for preparation of the antigen and several changes have been established in its processing with resulting improvements in its sensitivity and quality.

2. Salmonella Penetration through Shells of Fresh Chicken Eggs. In an experiment designed to study the penetration of Salmonella organisms through the shells of fresh chicken eggs, it was established that one or more eggs in all groups studied were penetrated by the Salmonella at the end of 24 hours, the time of earliest sampling. All eggs used in these studies had been penetrated by the organisms at the end of the sixth day.

(Athens, Georgia) (ADP a5-2(Rev.)

D. Pasteurellosis

At the National Animal Disease Laboratory, Ames, Iowa, studies have been conducted on 1) dissociation, 2) histopathology, 3) reservoirs of infection, and 4) characterization of antigens.

1. Dissociation. The genetic loss of ability of an avian strain of P. multocida to form a capsule resulted in a marked loss of virulence but not of immunogenic antigen(s) which was easily extracted with saline. There was also a direct relationship of virulence to colonial morphology.

A highly virulent culture from an acute case of fowl cholera produced fluorescent colonies which mutated in vitro and produced blue colonies. Organisms from the blue colonies also mutated and produced gray colonies. Organisms from blue colonies reverted in vivo and produced fluorescent colonies. Organisms from the fluorescent colonies occurred singularly or in pairs, were capsulated and virulent for chickens, turkeys, rabbits, and mice when administered via the mucous membranes of the upper air passages. Organisms from the blue colonies occurred singularly or in pairs, were non-capsulated and avirulent for chickens and mice, but virulent for rabbits and slightly virulent for turkeys. Organisms from gray colonies were non-capsulated, avirulent, and grew as a tangled mass of filaments. Organisms from the three types of colonies could not be differentiated by biochemical test or by serologic and immunologic response in chickens.

2. The Histopathology of Acute Fowl Cholera in Mature Chickens. Tissues from chickens that died from acute fowl cholera were compared microscopically with tissues from uninfected chickens to determine the histopathologic changes produced, and to characterize the type of tissue response in order to gain

insight on the cause of death. Lesions in the infected tissues were - generalized passive hyperemia; heterophilic infiltration of the lung, liver, adrenal, kidney, and thyroid; heterophilic depletion and hemopoietic cell degeneration in the bone marrow; generalized bacteremia, and acute focal necrotic hepatitis. Generalized passive hyperemia, the most pronounced and significant lesion observed, resulted from cardiac insufficiency, atony of veins and capillaries, or both, and is indicative of shock. The acute hemorrhagic enteritis commonly described in fowl cholera was shown to be a very severe acute passive hyperemia rather than an inflammatory reaction.

3. Fowl Cholera: Susceptibility of various animals and Their Potential as Disseminators of the Disease. Various animals were exposed to a culture of Pasteurella multocida isolated from a chicken dead of acute fowl cholera to determine their susceptibility to fowl cholera and their potential as carriers of the organism. Pigeons, sparrows, mice and rabbits died of acute septicemia when exposed intranasally. Rats, ferrets, guinea pigs, a sheep, a pig, and a calf failed to elicit any noticeable response to an intranasal exposure. There was a transitory temperature rise in a 7-week-old mink. The organisms were reisolated from the nasal passages of the calf and the pig 34 days after exposure and were still highly virulent for chickens. For this reason, other domestic farm animals may prove to be potential carriers of this disease.

One of 5 rats, 1 of 2 mink, and 11 of 19 mice fed viscera of chickens dead of fowl cholera developed a nasal infection, pneumonia and fatal septicemia, respectively. Since most of the animals tested were susceptible to or potential carriers of the fowl cholera organism, these experiments re-emphasized the necessity of a good sanitary program in the control of fowl cholera. The practice of feeding chickens dead of fowl cholera to other animals, or allowing domestic animals, free-flying birds or rodents to come in contact with dead or diseased birds, leads to a source of infection for neighboring or replacement flocks.

4. Isolation and Preliminary Characterization of an Antigen from Pasteurella Multocida which can induce immunity in chickens. It is generally recognized that whole cell preparations of the fluorescent mutant of Pasteurella multocida effectively immunize chickens against the same strain. It has been found that an immunizing agent can be consistently extracted with cold saline from the formalinized cells of the highly virulent fluorescent form, the relatively avirulent blue form, or a new avirulent chain-growing grey form. An immunogenic polysaccharide remains in the saline solution after extraction with an equal weight of phenol. It gave an absorption curve typical of a hexose in the phenol-sulfuric acid reaction and showed 0.8%N by the Kjeldahl method. Seven chickens were injected subcutaneously with the polysaccharide emulsified with Bayol F-Arlacel A, and challenged 19 days later with the virulent strain. All seven chickens were protected, whereas all seven controls died. (Ames, Iowa) (ADP a7-25)

Under a PL 480 Grant at the Veterinary Research Institute, Pulawy, Poland, research showed that high temperatures accelerate the mortality of chickens infected with fowl cholera. Tranquilizers seem to slow the rate of mortality in cholera-infected chickens. (E21-ADP-7)

E. Bluecomb Disease

In cooperative studies at the University of Minnesota, Minneapolis, an enterovirus and a bacteria of the genus Vibrio have been found quite consistently in the intestinal tracts of turkeys suspected of having bluecomb disease. In control turkeys, the enterovirus has not been isolated. However, an occasional Vibrio has been found. Pure cultures of either of these organisms alone, or mixtures of the two, when reinoculated into turkey poults, produced no observable disease. Rapid serial poult passage did not increase pathogenicity and protective immunity to bluecomb disease did not develop when such cultures were fed over a 4-week period.

Large numbers of Vibrio have been found in turkey poults showing bluecomb disease after being inoculated with bluecomb seed filtrates (milipore .22u) from which Vibrio could not be observed nor isolated. Vibrio have been found in the water from troughs watering infected turkey flocks, and have been found in chickens showing no symptoms of bluecomb disease. Vibrio isolated from swine with diarrhea caused no symptoms of bluecomb disease when inoculated into turkey poults.

Germ free studies and blood analysis, as well as other studies, are continuing toward determination of the possible relationship that Vibrio or the enterovirus may have with bluecomb disease of turkeys. (Minneapolis, Minn.) (ADP a5-19(C)

F. Newcastle disease

Studies at the National Animal Disease Laboratory, Ames, Iowa, showed that the immune response induced in chickens with two doses of a beta-propiolactone-killed Newcastle disease vaccine, administered subcutaneously at the second, and again at the twelfth week of age, prevented mortality, paralysis, and decrease in egg production after challenge with a virulent strain of Newcastle disease virus inoculated by either the intratracheal or intramuscular route. In unvaccinated birds, the intramuscular route of challenge produced greater mortality than the intratracheal route.

The virus neutralization capacity of serums of vaccinated birds correlated with the immune status of the birds throughout the experiment, for vaccinated birds had significantly high serum neutralization titers up to 70 weeks of age. The HI titers of vaccinated birds remained significantly high for only 50 weeks. Isolation of Newcastle disease virus 48 hours after challenge as an indication of the immune state of the bird was of no significance, since the virus was isolated from both vaccinated and unvaccinated birds after challenge. (Ames, Iowa)

Through cooperative research at the University of Maine, Orono, a Specific Pathogen Free (SPF) program has been conducted on broiler and breeder flocks during the past year, 1963-1964. A rigid set of standards for isolation and husbandry are required to conform to the program. A total of 23 SPF breeding flocks, comprising 143,337 birds have been on the program and have been found free of S_6 PPLO. Altogether 273,845 birds were tested for S_6 PPLO but many of these were not on the SPF program, and 5,845 samples were positive.

The program included a total of 68 SPF broiler flocks, totaling 1,324,648 birds which were followed from day-old chicks to processing in the plant. It was found that PPLO clean chicks, when placed on a clean farm, never became infected during the broiler-growing period of 9 weeks. Only killed Newcastle disease vaccine is used in this program. (Orono, Maine)

Cooperative research at the Wisconsin Agricultural Experiment Station, Madison, was directed toward -

1. Fundamental studies of Newcastle disease virus. With rare exceptions, all Newcastle disease virus (NDV) isolates that have been subjected to plaquing have been found to contain a mixed population consisting of 2 or more plaque types. It has been learned that the 2 plaque lines (S and L) derived from a single strain (Herts) differ in adsorption pattern, growth rate, virulence for chickens and antigenicity. Since these are most important properties, it is necessary to plaque purify all isolates before further characterizing them. Specific antisera must be prepared to the derived plaque lines rather than to the heterogenous wild strains. Two methods for calculating relatedness of antigens are being compared to determine the best procedure to use with Newcastle disease virus strains.

With the procedures based on plaque lines, it may now be possible to establish meaningful antigenic and pathogenic groups for epizootiological studies. In anticipation of such studies, the growth of NDV at aberrant temperatures and in cells of a wide range of species is being investigated.

2. Maintenance of the repository of strains of Newcastle disease virus. The study center for Newcastle disease virus was housed June 1, 1964, in a special laboratory in the new Veterinary Science Building. When the new lyophilizer arrives, the laboratory will be fully equipped. New strains have been added during the year; old stocks have been replenished and records have been up-dated.

3. International Symposium on Newcastle Disease. The proceedings of the International Symposium on Newcastle Disease as an Evolving Pathogen is now in proof. Copies are expected from the University of Wisconsin Press in late September. (Madison, Wisconsin) (ADP a5-18)

Research at the Veterinary Research Institute, Pulawy, Poland, under a PL 480 Grant, shows no change in the pathogenicity of Newcastle disease virus after 15 passages through 3-week-old chickens with artificially induced hypovitaminosis B_2 and B_{12}. (E21-ADP-2)

G. Avian Leukosis

Results of cooperative research on this problem at the Regional Poultry Research Laboratory, USDA, East Lansing, Michigan, will be reported by the Poultry Research Branch of the Animal Husbandry Research Division. (ADP a5-22)

H. Fowl Plague

Under a PL 480 Grant at the Instituto Jamie Ferran de Microbiologia, Madrid, Spain, research was conducted on fowl plague virus cultivated in cell cultures of chick embryos. The fowl plague virus cultivated in cultures of chicken embryo cells was inactivated with heat and combined with oil adjuvants prior to use for the immunization of chickens. Vaccines prepared in this manner seem to protect large numbers of animals from challenge with infective virus. (Madrid, Spain) (E25-ADP-1)

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AREA 6 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF FUR ANIMALS INCLUDING RABBITS

Problem. In the raising of fur animals in captivity, such as rabbits, chinchillas, mink, and foxes, disease problems incidental to the confinement of such animals are encountered. These include viral, bacterial, parasitic, mycotic, nutritional, and hereditary diseases. The enteric disease-complex causes great mortality in commercial rabbit production. It destroys whole litters and commonly attacks all susceptible rabbits on a farm. The respiratory disease-complex, perhaps, is second as a cause of mortality. In severe outbreaks over 50 percent of adult animals may die. These two diseases cause great economic loss to the rabbit industry, which produces an estimated 50 million pounds of meat annually and millions of dollars worth of rabbits for experimental purposes. Virus diseases of mink cause the greatest loss to the 7,000 mink ranchers now producing more than 5 million pelts annually valued in excess of $100 million. The role of helminths as carriers of rickettsial and viral agents causing, or associated with diseases of fur animals, is becoming of extreme importance and is one about which little is known.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving microbiologists and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of fur animals, including rabbits. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 3 professional man-years. This effort is applied as follows:

Enteric Disease Complex of Rabbits 0.5 at the U. S. Rabbit Experiment Station, Fontana, California, in cooperation with the Animal Husbandry Research Division, ARS.

Respiratory Disease-Complex of Rabbits 0.5 at the U. S. Rabbit Experiment Station, Fontana, California, in cooperation with the Animal Husbandry Research Division, ARS.

Coordinated Field and Laboratory Studies 1.0 at the U. S. Fur Animal Disease Research Laboratory, Pullman, Washington, in cooperation with the Washington State University.

Transmission of Infectious Diseases by Helminths 1.0 at the U. S. Fur Animal Disease Research Laboratory, Pullman, Washington, in cooperation with the Washington State University.

PROGRAM OF STATE EXPERIMENT STATIONS

Several State experiment stations are conducting research cooperatively with the Department on diseases of fur-bearing animals. Recent findings indicate that myxomatosis, a viral disease of rabbits, can be effectively controlled by the use of a modified live-virus vaccine. A twelve-year search for the cause and control of this disease culminated in this significant finding.

Scientists are attempting to provide useful information concerning the cause and control of Aleutian disease in mink. Transmission studies and a search for possible nutrient deficiencies or for toxins as factors in causing the disease are being made. Heredity as a cause also is being considered. Methods for prevention and treatment are being evaluated. Additional studies seek to identify the etiology of enteritis in mink and to develop methods for prevention and control.

The total State scientific effort devoted to fur-bearing animal disease research is 0.3 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Enteric Disease-Complex of Rabbits

In work at the U. S. Rabbit Experiment Station at Fontana, California, a vibrio-like organism and an infection of intestinal coccidia were believed responsible for widespread and excessive mortality in southwestern Oregon. Medication with sulfaquinoxaline and furazolidone was effective in curtailing losses and controlling the infection. Lower levels of aureomycin were found to be effective in increasing weights at weaning and lowering feed conversion. On a dollar basis, it was advantageous for the grower to feed a ration containing 20 grams of aureomycin per ton of feed. At this level no beneficial effect on mortality reduction was noted. (Fontana, Calif.) (ADP a6-5)

B. Respiratory Disease-Complex of Rabbits

An unusual manifestation of pasteurellosis has been noted during the work at the Fontana Rabbit Experiment Station. This infection produces thick and pendulous ears. Usually one ear is involved and the initial infection is believed to be the result of an injury or insect bite. Treatment with water soluble aureomycin appears to have been effective.

A survey of normal symptom-free animals indicated that 70% harbored pasteurella organisms and 16% harbored bordetella organisms in the nasal turbinates. Feeding of aureomycin and sulfamethazine, singly and together, had varying effects on the incidence. Aureomycin medication greatly reduced the incidence of nasal pasteurellosis in mature animals, but had little or no effect in the fryer-size animals. When the antibiotics and sulfa drugs were combined, a high incidence of gastro-enteritis was encountered. Because of the high incidence of enteritis and poor production, the use of this combination of drugs is not indicated. (Fontana, California) (ADP a6-6)

C. Field and Laboratory Studies of the Diseases of Fur Animals

Research workers at the Fur Animal Disease Research Laboratory, Pullman, Washington, submit the following report:

1. Transmission of Aleutian Disease Virus. An agent causing Aleutian disease (hypergammaglobulinemia) of mink has been transferred through 8 serial mink passages. This agent was present in the terminal stages of the disease in whole blood, serum, bone marrow, spleen, feces, urine, and saliva, but not in colostral milk. The virus can be transmitted *per os* in the form of feces or spleen and as an aerosolized spleen suspension.

2. A cause of False Negative Iodine Agglutination Tests. It is shown that mink in the advanced stages of the disease lose sufficient protein in their urine as a part of the nephrotic syndrome to render the iodine agglutination tests (IAT) negative.

3. Structural and Histochemical Observations of Liver and Kidney in Aleutian Disease of Mink. Results of detailed studies of selected significant tissue alterations in advanced Aleutian disease of mink have been obtained. A series of staining procedures and sequential histochemical techniques were directed toward an analysis of bile duct proliferation, renal tubular and glomerular degeneration and an arteritis accompanied by fibrinoid alterations. Associated with these changes was an intense plasmocytosis with the formation of Russell bodies. The combination of hypergammaglobulinemia, plasmocytosis and arteritis with fibrinoid necrosis suggested a possible hypersensitivity mechanism. Since this spontaneous disease of mink is most probably of viral etiology, the fact that it closely simulates some of the "collagen" diseases of man presents an intriguing system for further investigations.

4. The Familial Occurrence of the Chediak-Higashi Syndrome in Mink and Cattle. The occurrence of abnormal leukocytes in mink resulting from three types of matings was determined. From the mating Aa x aa, approximately one half (22/50) of the offspring showed the anomaly, and these 22 offspring were all identifiable as mink homozygous for the allele a for Aleutian coat color. Approximately one half of the offspring with the anomaly were male and one half female no matter which parent was the recessive in the mating, indicating that the trait is not sex linked. The homozygous dominant to homozygous recessive matings yielded no offspring with the anomaly and the homozygous recessive to homozygous recessive matings yielded all offspring with the anomaly. These are the expected results of a simple recessive trait.

All of the mutant color phases carrying the aa genotype possessed the leukocytic anomaly. Mink not having the aa genotype did not have the anomaly. The familial relationship of the cattle was studied. Sixteen Chediak-Higashi animals were born in the herd and all but three died. None of the cattle lived longer than four years. Abnormal leukocytes, the same as those observed in aa mink were found in Chediak-Higashi cattle.

5. Grey Diarrhea in Mink. Intensive treatment with vitamin B_{12} and folic acid failed to alter the course of idiopathic grey diarrhea in mink.

6. Mink Virus Enteritis. Fluorescent complement fixation tests revealed a cell culture panleucopenia variant (which will immunize mink against mink virus enteritis) is related to, or the same as, pathogenic panleucopenia virus. Inclusion bodies were found to occur in the nucleus of infected feline kidney cell monolayers. Using the acridine orange procedure, the presence of deoxyribonucleic acid (DNA) was revealed by a green fluorescence of the inclusion. (Pullman, Washington) (ADP a6-7)

D. Transmission of Infectious Diseases by Helminths

At the Pullman, Washington Fur Animal Disease Laboratory, studies on the persistence and transmission of viral and rickettsial diseases of helminths associated with diseases of fur animals were continued. Dogs recovered from Elokomin fluke fever maintained rickettsiae in their lymph nodes for at least 115 days. The metacercariae of the salmon poisoning fluke will persist in migrating Pacific salmon for at least four years. Complement-fixation tests have revealed that the disease known as 'salmon poisoning' may have a complex etiology which includes two entities. Ehrlichia canis may utilize the fluke Nanophyetus salmincola in an experimental situation. (Pullman, Washington) (ADP a6-8)

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AREA NO. 7 - MISCELLANEOUS INFECTIOUS AND NON-INFECTIOUS DISEASES OF ANIMALS

Problem. Included in this area of research are diseases such as vesicular stomatitis, which affects cattle, horses, swine, and man; poisoning by various plants, which differ in toxicity according to local conditions, and affect different species of animals in various ways; agricultural chemicals such as herbicides and pesticides, which may produce poisoning in animals, especially if not properly used, and may also leave dangerous residues in the soil, feed, or animal body; tumors, including cancer, which affect all species of animals; bloat, a common, serious condition in cattle and sheep; and potential dangers of "fall-out" from nuclear testing or attack. Investigations of these diverse hazards to livestock and poultry require modern techniques as well as fundamental approaches through chemistry, pathology, physics, physiology, and other scientific disciplines. The problems are so complex, diverse, and numerous that it has been impossible to more than scratch the surface in probing for basic knowledge required for protection of the nation's livestock and poultry populations.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, pathologists, physicists, and veterinarians engaged in both basic studies and the application of known principles to the solution of miscellaneous infectious and non-infectious diseases of animals. Research is being conducted at the designated locations.

The Federal scientific effort devoted to research in this area totals 30.0 professional man-years. This effort is divided among sub-headings as follows:

Incidence and Pathology of Tumors 1.0 at the National Animal Disease Laboratory, Ames, Iowa. A grant of PL 480 funds equivalent to $51,383 has been placed with the Veterinary Faculty, Ankara University, Ankara, Turkey, on etiologic investigation of bovine urinary bladder tumors due to enzootic bovine hematuria in Turkey and its relation to bovine paiplloma agent.

Vesicular Stomatitis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Components of Normal and Immune Serum 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Bloat in Ruminants 4.5 at the National Animal Disease Laboratory, Ames, Iowa, and through cooperative agreements with the California, Maryland, Mississippi, and Wisconsin Agricultural Experiment Stations, and with the New York State Veterinary College.

Preparedness for Diagnosis of Foreign Animal Diseases 0.5 at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York.

Toxicology and Pathology Related to Insecticides 2.5 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas, in cooperation with the Entomology Research Division.

Biochemical Effects of Agricultural Chemicals 0.9 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas, and through a cooperative agreement with the Stephen F. Austin College at Nacogdoches, Texas.

Detoxication Mechanisms in Cattle and Sheep 0.5 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas.

Cytological Responses to Antiparasitic and Other Agricultural Chemicals 0.5 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas.

Poisoning by Plants 1.1 at the Logan, Utah, Field Station, through formal cooperation with the Utah Agricultural Experiment Station, and informal cooperation with the U. S. Plant, Soil and Nutrition Laboratory of the Soil and Water Conservation Service, Ithaca, New York. A PL 480 grant of $56,746 was placed with the Instituto Biologico, Sao Paulo, Brazil, on The Study of Plants of the State of Sao Paulo poisonous to domestic animals.

Toxicity of Herbicides and Herbicide-Treated Plants for Domestic Animals 1.0 at the Logan, Utah, field station, with informal cooperation with the Utah Agricultural Experiment Station and the Crops Protection Branch of the Crops Research Division at Logan, Utah.

Alleviators and Diagnostic Tests for Plant Poisoning 1.0 at the Logan, Utah, field station through informal cooperation with the Utah State University, the Crops Research Division and the Forest Service.

The Susceptibility of Wild Animals to Foot-and-Mouth Disease 0.5 at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York.

Mycotic Diseases of Domestic Animals 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Investigations of the Genus Pasteurella 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Biological Changes Associated with Neuropathological Conditions in Animals 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Physiopathological Investigations of the Interrelations between the Respiratory, Circulatory, and Digestive Systems of Animals 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Proteins and Other Complex Molecules from Animal Disease Agents Derived Primarily from Surface Structures and Extracellular Products 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Chemical and Physical Studies on Microbial Antigens 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Physiology of Normal Mammalian Cells Grown in Tissue Culture 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

PROGRAM OF STATE EXPERIMENT STATIONS

Principal emphasis in this area is being placed on problems related to poisoning of livestock due to toxic plants and agricultural chemicals. A number of States are isolating and identifying the toxic principals involved in major poisonous plants. The pharmacological action of these compounds in animals is being evaluated and antidotal measures being developed. Other research is aimed at the toxicology and metabolic fate in animals of insecticides, nitrates, urea, selenium, molybdenum and fluorides.

Basic work is in progress at a number of locations on bloat in ruminants. Factors which influence formation and elimination of ruminal gases are being determined and the physiological processes of the animal which contribute to bloat susceptibility are being assessed. An evaluation is being made of the soil-plant-animal interactions which contribute to the bloat problem. Bloat preventive measures are being developed and evaluated at several locations.

The total research effort on miscellaneous diseases of animals at the States is 17.7 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Components of Normal and Immune Sera

At the National Animal Disease Laboratory, Ames, Iowa, separation studies by density ultracentrifugation in sucrose gradients have been conducted on serums from heifers after exposure to viable Brucella to determine changes in the concentration of high and low molecular weight Brucella agglutinins with time after exposure to Brucella. The results of these cooperative investigations are reported under Line Project ADP a1-3 - Investigations of Brucellosis of Cattle. (Ames, Iowa) (ADP a7-14(Rev.)

B. Bloat in Ruminants

In cooperation with the California Agricultural Experiment Station at Davis, the investigation has been directed toward study of metabolic processes in anaerobic systems and the development of new techniques for the characterization of rumen metabolism under various conditions including bloat. The

mechanisms of electron transport associated with the production of volatile fatty acids in several anaerobic species were characterized. The possibility that enzymatic criteria can be employed effectively in the characterization of changes in the rumen microflora on various diets was investigated and data presented indicate that this is a useful technique. (California)

At Cornell University, Ithaca, cooperative studies revealed that an increase in blood flow to the rumen was seen upon feeding. This increase continued beyond the act of feeding, and was independent of weight-volume changes in the rumen, and of motility change. Such increase could not be ascribed to increased output from the heart. Feeding under conditions where rumen fermentation was minimal limited the increase in blood flow to the period when the animal was eating. Two fermentation products prominent in the rumen, carbon dioxide and butyric acid, were shown to be important regulatory chemicals for the blood flow increase, in concentrations available normally in the rumen of cattle following feeding. (New York)

In cooperation with the Maryland State Agricultural Experiment Station, at College Park, the saliva of the ruminant has been shown to have bloat preventing properties. The mucin and perhaps other components are involved, thus the interest in saliva research. In the present experiments, a relationship between the susceptibility to bloat and salivation was lost in the wide day-to-day variation. This variation was attributed to the water conent of the feed. As the water content of the feed increased, salivation decreased.

Other work has shown that there are active bloat promoting component(s) of the legume plant. Attempts to isolate these factor(s) have failed to date.

This research, hopefully, will eventually provide animal breeders with a genetic-test for resistance to bloat, and plant breeders with a genetic-test to produce non-bloat causing legumes. A few years ago these suggestions appeared a little unrealistic, but today enough data are available to enable sincere predictions. (Maryland)

At the Mississippi State Agricultural Experiment Station, State College, during the 1963 spring grazing season, 10 calves were grazed on crimson clover for about 25 days. Only two calves bloated a total of 20 times out of a possible 100 times and there was no severity greater than moderate bloat. The two "bloaters" and two steers which did not bloat, were used for obtaining samples of rumen content. The rumen content was analyzed for volatile fatty acid content, using a gas Chromatographic technique.

These data indicated that the bloater steers had a higher volatile fatty acid (VFA) concentration per ml of rumen fluid during the bloat-producing stage of forage growth than the same bloater steers during the non-bloat-producing stage of forage growth. In the non-bloater steers rumen fluid VFA concentration did not change appreciably during the two periods of forage growth. The VFA concentration of rumen fluid from the bloater steers during the pre-bloom stage of forage growth was higher than the non-bloaters. However,

during the full-bloom stage of forage growth, the VFA concentration of the bloater steers was lower than the non-bloater steers. These variations in VFA concentration indicate that perhaps the bloater steer metabolizes the bloat-producing forage at a faster rate than the non-bloater steer.
(Mississippi)

Cooperative work was continued with the Wisconsin Agricultural Experiment Station, Madison, where experiments have been carried out to determine why ruminants feeding on hay or grass will rarely bloat, but may do so readily when fresh, rapidly grown alfalfa mainly constitutes the diet. Recent evidence along these lines point to a correlation between the increased viscid ruminal contents during bloat and the available pectin methyl esterase (PME) activity in forages consumed by the ruminant animals. Bloat-regulating substances administered to and operating in digestion of ruminants are currently being studied, as follows:

a) Pectin methyl esterase activity was extracted from alfalfa, hay, and grass with buffered sodium chloride (BSC), centrifuged ruminal fluid (CRF), and with strained ruminal fluid (St. RF). The level of PME activity was measured by titrating with a standard base the increase in acidity of a reaction mixture containing PME and pectin. Alfalfa extracts in the medium most closely resembling ruminal contents -- St. RF -- contained far more PME activity than those of hay and grass. The differences between PME activity of alfalfa extracted with BSC and CRF were not as great; grass extracts always contained the least amount of PME activity. Thus in the ruminant animal, alfalfa may release more PME activity than either hay or grass. Since PME is considered to be an important factor in pasture bloat, animals feeding on the fresh, rapidly grown alfalfa may be more likely to bloat than those consuming hay and grass.

b) Pectin methyl esterase activity of fresh alfalfa extracted with active (unheated) St. RF was greater than the PME extracted with heated St. RF. Active extracts from frosted alfalfa incubated in St. RF contained more PME activity also than that in St. RF preparations of frosted birdsfoot trefoil and bromegrass; only a mixture of this alfalfa extract and pectin produced a soft gel after several hours.

c) Alkyl aryl sulfonate sodium incorporated in a mixture for delayed release was effective in controlling the digestive processes of legume bloat when each animal's intake was carefully assured. Moldy feed should be avoided since it interferes with the intake.

d) Alkyl aryl sulfonate (AAS) also reduced the activity of PME in water, 0.1 M NaCl and in ruminal fluid media. Again a gel formed in those AAS uninhibited reaction vessels where pectin and PME were incubated only in centrifuged ruminal fluid (cf. a)above). Alkyl aryl sulfonate seemed to be a non-competitive inhibitor of PME activity in water when the kinetic data were analyzed.

e) An inhibitor to PME gelling of pectin in presence of Ca ions was demonstrated in strained ruminal contents (SRC) or fluid (St. RF) and in the precipitate (Ppt.) from SRC. Diluting SRC decreased the amount of inhibition to the gelling of pectin by PME; heating destroyed the inhibition. As a comparison AAS also inhibited PME gelling of pectin.

f) Initial exploratory experiments suggest that alfalfa extracted with St. RF may also contain more available lipase, protease, amylase, and cellulase activities than the corresponding extracts of birdsfoot trefoil and of bromegrass. (Wisconsin) (ADP a7-15)

C. Toxicological and pathological effect of insecticides, herbicides, fungicides and other agricultural chemicals on livestock and poultry

At the Division's Toxicological Investigations Laboratory, Kerrville, Texas, the following research studies were conducted:

In work on insect chemosterilants, Jersey heifers were selected and divided into groups for treatment and controls. All were observed for 3 months to establish estrous cycles, then the principals were fed apholate daily at a dosage of 1.0 mg/kg. No effect of apholate upon the estrous cycle of the heifers was apparent at the end of 7 months. The heifers were then placed with a Hereford bull for breeding, the apholate feeding continuing at the same dosage. Effects upon implantation of the embryo and upon gestation are currently being observed.

A test was completed with a single survivor of a group of 4 sheep given 1.0 mg/kg of apholate daily. The test feeding was terminated after the sheep survived 759 daily doses. Principal effect of apholate on this sheep was a reduction of white blood cells and blood platelets. Recovery from these deficiencies have been very slow and is still under study.

Ewes and rams were placed on a diet containing a dosage of 0.5 mg/kg of apholate and were bred during the feeding period. Ovarian and testicular biopsy tissues did not show evidence of damage by apholate. The ewes lambed normally. White blood cell numbers were slightly reduced. The test was terminated after 494 daily doses had been administered.

A second study was designed to show hematologic and teratogenic (deformity producing) effects that might occur with the feeding of apholate. Rams and ewes were selected, placed on diets containing a dosage of 1.0 mg/kg of apholate and allowed to breed. Three of four test ewes, and both control ewes, delivered normal lambs. One test ewe delivered a deformed lamb. The deformed lamb showed a lack of eyes and eye nerves, nose, and snortened upper jaw. There was no spleen and the liver was rudimentary in size. A mass outside the body resembled liver. The dam of this lamb had received approximately 189 daily doses of apholate at the time of conception and the lamb was delivered after 345 daily doses had been given. This study will continue through another gestation period.

In studies on insecticides, the response of Brahman cattle to Ciodrin, coumaphos (Co-ral), dioxathion (Delnav) and Compound 4072, was compared to the responses of cattle of Hereford or other European breeding. Each of the four compounds produced a different result. Coumaphos and Ciodrin produced greater blood cholinesterase depression in Brahman cattle than in cattle of other breeding; Compound 4072 and dioxathion had just the opposite effect.

A new organic phosphorus compound containing bromine as well as chlorine has been introduced by the Stauffer Chemical Company and is worthy of special mention. This compound, Bromophos, has the lowest toxicity to sheep and cattle of any of the organic phosphorus compounds, or chlorinated hydrocarbons, we have studied during the past 17 years. If its effectiveness and residue data prove favorable, this compound may be a break-through in the development of compounds having a maximum margin of safety.

Twenty-one new insecticides being explored for usefulness by the Entomology Research Division were given preliminary toxicologic study in young dairy calves during this fiscal year. Those compounds, identifiable only by assigned numbers, have no special significance at this time. The data derived will guide the entomologists in their further consideration of these compounds as livestock treatments.

Thirteen compounds that have passed preliminary screening for toxicity in this and the preceding fiscal years were applied to mature cattle as sprays, "pour-ons", or administered orally, all by entomologists. The staff observed the reactions of the animals and suggested adjustment of dosage when this was necessary. Other compounds were administered to sheep by entomologists with the Division staff members as observers.

Herbicides are, with increasing frequency, being suspected as the cause of poisoning in livestock and poultry by veterinarians confronted with sick animals under less than obvious circumstances. This laboratory has continued its studies of the toxicity of a large number of commercially available herbicides of the synthetic variety. The general conclusion reached in our studies is that most of these compounds are not hazardous unless used in the most careless way possible. A few have shown sufficient toxicity to indicate a hazard should they be used with only a little neglect. A total of 18 herbicides was studied in sheep and 5 were studied in chickens.

Research has continued on the treatment of animals poisoned by organic phosphorus compounds. Various oximes have been studied for their effectiveness alone or in combination with atropine. Pralidoxime chloride (Protopam chloride) has shown good effectiveness alone and in combination with atropine, particularly when the dosage of pralidoxime is kept high and is repeated. TMB4, a relatively new compound, has given good results in the treatment of coumaphos (Co-ral) poisoning, the most difficult, usually, to control.

(ADP a7-23)

Analytical procedures of proper sensitivity have not been available for 2,4-D and 2,4,5-T in animal tissues. Carbon 14-labeled 2,4-D and 2,4,5-T were utilized this year to guide the design and prove the efficacy of the numerous steps required to successfully provide a sensitive analytical procedure.

Tritium, a radioactive isotope, is used to provide a source of electrons in detectors operating on the principle of electron capture by various organic compounds passing through a gas/liquid chromatograph. Such detectors provide accurate sensing of compounds present in amounts of 1-100 nanograms (1/28 of a trillionth of an ounce) in the sample injected into the instrument - or parts per trillion in meat or milk. Two instruments utilizing these detectors were purchased. One has been in service about 8 months, the other has not been received. Monitoring of the tritium source for leakage and other radiological hazards has been duly accomplished.
(Kerrville, Texas) (ADP a7-12)

D. Biochemical effects of agricultural chemicals and control substances in cattle and sheep

At the Division's Toxicological Investigations Laboratory, Kerrville, Texas, research studies were made as follows:

Silvex. Orally administered silvex produced a 2-fold increase in the activity of serum glutamic-oxalic transaminase of sheep 24 hours prior to death. An increase of serum lactic dehydrogenase was noted in the same sheep, with a possible shift in the relative percentage of the isozymes composing this enzyme. These enzyme changes did not occur in sheep treated in the same manner but which failed to show poisoning.

Coumaphos (Co-ral). A study to determine the interactions of Vitamin A and phenothiazine/lead arsenate drenches with coumaphos (Co-ral) and with contaminated coumaphos was reported for fiscal year 1963. A number of interactions resulting in an increased toxicity of the insecticide were noted in that report. During fiscal year 1964 studies on blood from those animals included the effects on the Vitamin E of plasma and the Vitamin A and carotene of plasma.

There was no significant difference between treatment groups for Vitamin E or for carotene. Vitamin A and carotene values decreased throughout the test in all groups. Plasma Vitamin A was affected by two interactions of treatments. With contaminated coumaphos (Co-ral), animals fed normal diets had lower mean values than those fed additional Vitamin A, whereas those animals treated with normal coumaphos showed no differences in plasma Vitamin A, whether supplemented with A or not. In animals treated with normal coumaphos the plasma Vitamin A was increased by drenching with phenothiazine/lead arsenate. In those cattle treated with contaminated coumaphos the plasma Vitamin A was lowered by drenching with phenothiazine/lead arsenate.

As a preliminary essential to further studies, normal activity values were determined for the following blood enzymes in sheep:

Serum glutamic-oxaloacetic transaminase: mean activity was found to be 61 units/ml., with a range of 45-77 units/ml.

Serum glutamic-pyruvic transaminase: mean activity was found to be 11 units/ml., with a range of 5 to 17 units/ml.

Serum lactic-dehydrogenase: Total activity of this enzyme gave a mean of 790 units/ml., with a range of 598-982 units/ml. Of the total activity, isozyme 1 accounted for 61%, with a mean activity of 484 units per milliliter, ranging from 290-678; isozymes 2, 3, and 4, combined, accounted for 31% of the total activity, with isozyme 5 accounting for 8% of the total. (Kerrville, Texas) (ADP a7-18)

Cooperative work was continued at the Stephen F. Austin State College, Nacogdoches, Texas, toward the development of a particle size spectrometer. The range of an Ionovac speaker has been extended to 140 KC and the range of the Ionovac microphone extended to 58 KC. The mechanism by which sound is produced by ionized air is under study with a view toward increasing the power handling capacities of both the speaker and the microphone. Preliminary calculations of drop size based on the change of sound velocity in an aerosol gave very good agreement with the drop size determined photographically.

In other research at this location, solubility studies on potassium antimony tartrate have been made at several temperatures. The solubility of barium chloride in saturated solutions of potassium antimony tartrate has been measured at several temperatures. Apparently two solid phases can exist in this system. The composition of each has not as yet been determined. (Nacogdoches, Texas) (ADP a7-18)

E. Investigations of Detoxication Mechanisms in Cattle and Sheep

At the ADP Division's Poisonous Plant Laboratory, Logan, Utah, sheep were fed 2 grams of 2,4-D daily for 30, 60, or 90 days. The sheep were slaughtered one day after the last dosage (30, 60, or 90) and tissues were collected and shipped under refrigeration to Kerrville, Texas, for analysis. Samples were analyzed by gas/liquid chromatography at a sensitivity that would detect residues of 0.05 ppm or greater. Residues of 2,4-D were less than, or equal to, control values in fat and less than 0.05 ppm in liver. In muscle, the range was from less than 0.05 ppm to a maximum of 0.16 ppm. Residues in kidneys ranged from less than control to 0.8 ppm. The kidneys were the only tissues in which the residues detected increased with the length of the feeding period. Since 2,4-D is eliminated almost entirely by the kidneys it is probable the 2,4-D in the kidneys represented the chemical in transit rather than a true deposit in the tissues. This study indicates there is virtually no tissue residue problem in animals consuming 2,4-D contaminated forage, even when the dosage ingested is excessively high and prolonged far longer than could occur under practical conditions of plant treatment.

Atropine, the standard therapeutic agent against poisoning by organophosphorus compounds, acts by opposing the stimulation resulting from accumulation of acetylcholine but does nothing to treat the basic biochemical lesion, the inhibition of the essential enzyme, cholinesterase. A need for an antidote that would reactivate inhibited cholinesterase has been recognized for many years. Various oximes have been proposed and have shown beneficial action together with specificity toward both compounds and species of animal. In previous studies, the oxime dosages employed did not seem useful against coumaphos (Co-ral) poisoning.

A new oxime, 1,1'-trimethylene bis-(4-hydroxyiminomethyl)-pyridinium bromide, (TMB4), has been considerably more effective than previously studied oximes in preventing death and hastening recovery of coumaphos-poisoned cattle.

Pralidoxime as 2-PAM chloride (Protopam chloride), in high dosages, this year gave encouraging control of coumaphos poisoning.

Although carbamate insecticides inhibit cholinesterase, as do organic phosphorus compounds, the process is by carbamylation instead of phosphorylation. Laboratory animal studies indicated that oximes such as 2-PAM (Protopam chloride) intensified the action of Sevin instead of reversing the enzyme inhibition. Phenothiazine derivatives have some potentiating effects in organic phosphorus insecticide poisoning. Cattle were poisoned by Sevin, then treated with 2-PAM chloride and Promazine. Clinically, the signs of intoxication were markedly increased after the administration of the two drugs, indicating a potentiating effect of one or both.

This laboratory has been active since 1950 in demonstrating the extreme significance of the effects of formulation in the spraying and dipping of livestock. In cooperation with the Entomology Research Division, performance standards were established for emulsions, but not for suspensions.

Through the years, manufacturers of wettable powders of lindane, as used in dips for scabies, tended to produce a single type of lindane powder, intended for plant use. Ultimately, lindane had to be removed from the federally approved list of scabicides because these powders were rapidly depleted from dips and could not be trusted to provide kill of the scabies mites. The specific toxicity of lindane for the mites has not changed.

The Albuquerque, New Mexico, Laboratory of the ADP Research Division is careful in its evaluation of new scabicides to include vat analyses, performed by the Kerrville Laboratory, to determine depletion or increases of concentration of the scabicidal chemical. During this fiscal year, analyses of dips made with coumaphos (Co-ral), ronnel (Korlan) and Shell 4294 (Ciodrin) were performed. Ronnel, as Korlan, performed extremely well, maintaining its concentration precisely during the dipping of 65 sheep in a 600-gallon vat. Shell 4294, or Ciodrin, was a complete failure, the concentration being reduced by more than 60% by the passage of 52 sheep through a 700-gallon vat.

Coumaphos, as Co-ral, showed an essentially uniform tendency to increase in concentration, indicating that sheep's wool was selectively absorbing more water than toxicant; 56 sheep were dipped in a 700-gallon vat.
(Logan, Utah) (ADP a7-19)

F. Cytological responses to toxic actions of antiparasitic and other agricultural chemicals in cattle and sheep tissues

At the Division's Toxicological Investigations Laboratory, Kerrville, Texas, it was found that the potential value of insect chemosterilants for the control of insects is, as yet, unlimited. Such powerful compounds must be restricted to use by qualified people who have been provided with full knowledge of the potential hazards associated with them. Although none of these materials can be obtained publicly - nor has a decision been reached concerning the one, or ones, of the 50 or more known compounds to be employed, the studies at this Laboratory have continued, in dairy cattle and in sheep, to determine the hazards to livestock. Previous reports have emphasized the radiomimetic effect produced by apholate, tepa, and metepa, particularly the deleterious effect upon the tissues that form white blood cells. This year a second effect, teratogenesis - that is, the production of monstrosities and defects in the young of animals and birds - has been demonstrated.

In incubating chicken eggs injected with apholate, tepa, or metapa, at various times, a disconcerting number of defective chicks were produced by very low, realistic, exposures to these three compounds. Defects included shortened upper or lower beaks, crossed-beaks, absence of legs, curled and fused toes, herniation of the brain, lack of eyes, schistosomus, and growth retardation. At high dosages the embryos died or did not begin development.

A lamb was born to a ewe fed apholate and showed a total lack of eyes and eye nerves, a lack of upper jaw and nose, and numerous other anatomic defects, not the least of which was a total failure to develop a spleen. Dairy cattle have been put into test to determine the effects of these compounds.

One should not construe these findings as indicating a hazard incompatible with the use of chemosterilants. The significance of these studies lies in the demonstration of the hazards attendant upon their use in an uncontrolled, over the counter, way. (Kerrville, Texas) (ADP a7-20)

G. Poisoning by Plants

1. Cyclopian-Type Malformation in Lambs. At the Division's Poisonous Plant Research Laboratory, Logan, Utah, it has been found that a congenital cyclopian-type malformation in lambs has been occurring in bands of range ewes in Idaho for many years. The deformity has occurred each year affecting from 1 to 25% of the lambs born in individual bands. The condition has shown a wide variation between bands and from year to year. It was always considered to be a hereditary problem causing unavoidable losses.

Research workers of the Animal Disease and Parasite Research Division at this Laboratory found the deformity to be caused by the maternal ingestion of Veratrum californicum, a poisonous range plant. It was found that grazing the sheep on Veratrum-free ranges for the first 30 days of the breeding season, or until after the first killing frost, would prevent the deformity in the lambs. This practice has been carried out and has almost eliminated the occurrence of cyclopian-type malformation in the lambs from range ewes.

The experimental work carried out this year has shown the malformation occurs when the Veratrum plants are eaten by the ewes on the 14th day after breeding. Ingestion of large quantities of the plant before or after the 14th day may cause the embryos to die, but does not cause abnormal development.

In cooperation with Division research workers at the National Animal Disease Laboratory, the teratogenic material from the plant has been extracted by an ethanol extraction of the plant residue remaining after an initial benzene extraction in the presence of ammonia. A 250-fold purified preparation from this extract also produced malformations. Because of the extractability and solubility of the active material in various solvents, the biological properties and certain structural considerations, the first premise has been that one or more of the Veratrum alkaloids would prove to be the teratogen. Experimental work thus far supports this premise and tends to limit the possibilities among the various compounds of the Veratrum alkaloid class. Experimental data suggests that the teratogenic material may be a glycoside or parent alkamine of the Veratrum alkaloid class.

A pilot study to determine if the mechanism in cyclopia-type malformation in lambs caused by ingestion of Veratrum californicum is of chromosomal origin was started. The mechanism in which the teratogenic agent in Veratrum californicum causes the deformity in lambs has only been investigated in a preliminary manner to establish techniques. Several theories have been advanced, but the first to be studied is the effect of the teratogen on the chromosomes in the cells of the deformed lambs. Considerable time and effort has been put into working out a technique to study the chromosomes in sheep, as the standard method of using white blood cells could not be adapted to sheep's blood. As this report was made, a technique was finally developed by tissue culture procedures to study the chromosomes in kidney and testicular tissue. The exact number and individual types of chromosomes in sheep are not as well established as in other species of animals. Studies indicate a possible total of 52, with some possible aberrations present.

Feeding Veratrum californicum to cattle did not cause congenital deformities, but resulted in profuse vomiting and loss of appetite. It is uncommon to observe cattle grazing this plant under range conditions. The plant seems to be unpalatable for cattle but very palatable for sheep.

2. Crooked Calf Syndrome. At the Division's Poisonous Plant Research Laboratory, Logan, Utah, research workers report that for many years the crooked calf syndrome has been prevalent on various range areas throughout the western States and Alaska. The seasonal incidence of this disease varies. However, mortality and morbidity rates from this disease cause livestockmen large annual economic losses.

Varied opinions and thinking exists relative to the manifestations, pathogenesis and lesions associated with the crooked calf syndrome. There is need to characterize this disease and determine the lesions associated with it. To date abnormal tendon flexure, dwarfisms, spastic lethals, malocclusions (another achondroplastic condition) and internal hydrocephalus has been confused with this congenital malformation.

The feeding of Lupinus sericeus and lead acetate during early stages of gestation has caused congenital deformities and abortion in cattle. Feeding lupine without lead acetate in early stages of gestation caused abortion of a malformed calf. Lesions of the experimentally produced congenital deformities associated with lupine during early stages of gestation are characteristic of those associated with clinical field cases of the crooked calf disease.

Congenital malformed calves somewhat characteristic of the crooked calf syndrome have resulted from the mating of Hereford cows to a specific Hereford bull. Based on facts and information to date, it appears that this condition may be hereditary. The lesions of the calves from this specific mating needs additional characterization for comparison with those of the crooked calf disease. Additional study and work is necessary to determine the etiological factor and/or factors of this specific type of congenital malformation.

The matings of a congenital malformed bull to congenital malformed cows resulted in normal offspring. To date, there is no indication that this form of the crooked calf syndrome is of a hereditary nature.

Based on data and information accumulated to date, it appears that the crooked calf disease is associated with the dam ingesting some toxic substance during the early part of gestation, or some interference with normal trace mineral metabolic processes. The seasonal incidence of this disease appears to be associated with the variation of climatic conditions that influences the availability of feed on the range, types of plants on the range, and the degree of dryness of the range. Change in herd management, such as proper mineral supplementation and delayed breeding (1 month) of cows has also reduced the incidence of the disease in some cases.

The feeding of Lupinus sericeus to pregnant rabbits and guinea pigs did not adversely affect their offspring. This necessitates the continued use of cattle for experimental studies on the crooked calf syndrome.

Many livestockmen think the crooked calf syndrome is hereditary and have disrupted and hindered their ranching operations by selling valuable bulls that sired malformed calves. Other livestockmen have altered their grazing programs with resultant decreased range utilization because they have had congenital malformations associated with grazing range areas infested with lupine.

If the cause, pathogenesis, and prevention of the crooked calf syndrome can be determined, it could save livestockmen large annual economic losses and be valuable in medical and related professions in helping to gain additional insight to some aspects of disease processes.

3. Experimental Feeding of Loco Plant. Locosis or loco poisoning continues to be a serious problem in many areas of the western States. This condition has been prevalent on various range areas for many years. The seasonal incidence of the disease varies. However, mortality and morbidity rates from the disease cause livestockmen large annual economic losses.

The feeding of loco plant (Oxytropis sericea) caused abortion of abnormal feti when fed for 15 and 17 days during various stages of gestation. The aborted feti were moderately macerated and had skeletal malformations and malalignment of limbs. Some of the pregnant ewes fed 227 grams of loco plant (Oxytropis sericea) daily evidenced symptoms typical of locosis in 15 days. As the animals remained on treatment, the symptoms became progressively worse.

Loco plant was fed to pregnant rabbits and guinea pigs. Based on the results of this loco experiment, one can conclude that Oxytropis sericea, when fed in early gestation at the rate of 325 mg per pound of body weight, does not adversely affect the offspring of rabbits or guinea pigs.

A number of locosis animals were observed on the range. The symptoms of the clinical field cases were characteristic of those experimentally produced. There was a decrease of food intake, loss of body weight, weakness, muscle tremors, irregular gait, incoordination and evidences of a central nervous system disorder.

If the pathogenesis and prevention of locosis could be determined, it would save livestockmen large annual economic losses. Findings would be valuable in medical and related professions in helping to gain additional needed insight to the abortions and disease processes associated with locosis.

4. Oxalate Toxicity in Sheep. Oxalate poisoning in sheep (Halogeton glomerata and Sarcobata veniculata) is one of the principal causes of losses to the sheep industry in the Intermountain area during the fall, winter and early spring months of the year. Every year several large losses of sheep are reported. For example, this year one man is reported to have lost 1300 ewes while another lost 450 head. There were also many smaller losses reported. These sheep were all carrying nearly a full fleece of wool

and would have lambed a short time later. The losses due to the consumption of sublethal amounts of these plants are unknown. Studies have been initiated to gain a more basic understanding of oxalate poisoning in sheep.

This year in vitro fermentation studies, using rumen contents and oxalates have indicated that oxalates may markedly decrease cellulose digestion in the rumen. These studies also indicated that if the sheep from which the rumen content is obtained was fed some source of oxalates 4-7 days prior to the in vitro fermentation the decrease in cellulose digestion was not so great and there was destruction of the oxalate ion. There was no destruction of oxalates by rumen content from a sheep that had not been fed a source of oxalate. The principle function of the rumen is the digestion of cellulose. One of the principle sources of energy to the ruminant animal is from the digestion of cellulose.

In vitro studies using various calcium and magnesium supplements with oxalates have been carried out. The rate or fermentation of cellulose was used as a measure of the value of the supplement in counteracting the depressing effect of oxalate on cellulose digestion. Dicalcium phosphate, calcium carbonate, calcium chloride and magnesium sulfate were all of value in removing these depressing effects while bone meal was of less value. These same studies indicated excessive amounts of calcium may have a detrimental effect on cellulose digestion. (ADP a7-7(Rev.)

H. Alleviators and Diagnostic Tests for Plant Poisoning, and Methods to Avoid Harmful Residues in Animal Tissues for Ingesting Chemically-Treated Plants. Sheep fed 2 grams of 2-4D in acid form daily for 30, 60, and 90 days, did not cause any clinical signs of toxicity, no apparent tissue residue, or any discernible histopathological tissue changes. The amount of 2-4D ingested by each animal daily was in excess to what it would receive if fed forage sprayed with the herbicide. This information substantiates the safety of 2-4D when ingested by sheep. One of the most common herbicides used on the range, farm, and around the home, is 2-4D. All information available indicated it is safe to use on any forage that may later be eaten by livestock. (Logan, Utah) (ADP a7-17)

I. Mycotic Diseases of Domestic Animals

Research workers at the National Animal Disease Laboratory, Ames, Iowa, report as follows: Cutaneous Streptothricosis is an infectious disease of cattle, horses, and goats. Closely allied infections are seen in sheep resulting in "lumpy wool" (mycotic dermatitis) and "strawberry foot rot." Prior to 1961 Cutaneous Streptothricosis had not been reported in the United States. Recent reports have indicated the presence of infection in deer and horses in New York, and in cattle in Texas, Iowa, Kentucky and Kansas.

Dermatophilus congolensis, the causative organism of Cutaneous Streptothricosis, has been isolated in this laboratory from clinical specimens taken from naturally infected cattle in Iowa and Kentucky. The organism

grows slowly and is readily overgrown by contaminants in the clinical specimen. No selective mediums have been described to facilitate isolation. A presumptive diagnosis may be made, in the absence of cultural isolation, by demonstrating typical forms of Dermatophilus in stained smears of exudate specimens. However, specimens from certain cases may be very poor in diagnostic forms and an extremely diligent search of the stained film is necessary to locate such forms.

Methods to facilitate cultural isolation and examination of exudate smears have been developed. These methods included the adaptation of fluorescent antibody techniques for the specific identification of the organism in exudate smears. Application of the fluorescent antibody technique greatly facilitated location and identification of the causative agent in these preparations. Application of fluorescent antibody to smears of 4 different culture strains of D. congolensis indicated similarities of antigenicity of the 4 strains.

Cultural isolation has been facilitated by a differential filtration procedure. Exudate specimens ground in the presence of sterile saline are passed through a membrane filter and the resulting filtrate is cultured. The application of these two methods has permitted identification and isolation of D. congolensis where other methods have failed.

(Ames, Iowa) (ADP a7-24)

J. Biological Changes Associated with Neuropathological Conditions in Animals

At the National Animal Disease Laboratory, Ames, Iowa, investigations of biochemical and functional changes in the central nervous system of sheep associated with induced convulsions involved the development of surgical procedures for permanent cannulation of the cerebrospinal fluid space at the base of the brain and cannulation of the carotid artery. These cannulae permitted the continuous monitoring of pressure changes as well as the taking of samples of cerebrospinal fluid and blood serially for biochemical analysis during experiments in which convulsions were induced by the administration of various compounds. These included carbon dioxide, insulin and heptachlor, a convulsant insecticide. Criteria measured during the convulsive seizures included blood pressure, heartbeat, respiration, electrocardiogram, and cerebrospinal fluid pressure. Biochemical determinations of blood and cerebrospinal fluid included glucose, transaminase enzymes, lactic dehydrogenase enzyme, proteins, and the electrolytes sodium, potassium, calcium and magnesium. The convulsive seizures were accompanied by changes in many of these criteria; however, there were differences, depending on which compound was used to induce seizures.

Investigations of pharmacologic effects in sheep and goats of alkaloids and extracts from the plant Veratrum californicum have been initiated. This plant has been previously shown by workers at the ADP Research Division Laboratory, Logan, Utah, to cause malformations of the central nervous

system in lambs when eaten by the ewe. Two previously unreported effects were found: 1) certain purified alcoholic extracts of the plant, when continuously infused intravenously into sheep and goats, produced a 3-7 fold increase in blood sugar (glucose). This effect was noted in both intact and totally adrenalectomized animals. 2) this same plant extract, when given in high doses, caused a complete cessation of the electrical activity of the crebral cortex of the brain. Artificial respiration, using oxygen, caused a reversal of this effect and complete recovery of the animal.

Fractions and pure alkaloids from this alcoholic extract did not produce these effects. Commercial alkaloids, extracted in a different manner from another plant, Veratrum viride, that are used in medicine as agents for lowering blood pressure were also studied. They had a marked stimulatory effect on the gastrointestinal tract of sheep, causing retching, vomiting, and expelling of rumen gases. These observations have led to the initiation of a study of the potential of these compounds for the relief of bloat in the ruminant.

A surgical procedure has been developed to permit total bilateral removal of the adrenal glands from sheep and goats simultaneously through a single incision. These animals are then maintained by the daily administration of adrenal cortico-hormones. This enables the researcher to study carbohydrate metabolism without the interference of the "fight-or-flight reaction" of the adrenal gland, which causes an elevation in blood sugar.

(Ames, Iowa) (ADP a7-26)

K. Physiopathological Investigations of the Interrelations between the Respiratory, Circulatory and Digestive Systems of Animals

At the National Animal Disease Laboratory, Ames, Iowa, the work performed on this project included 1) the finding that microorganisms are transported from the rumen to the respiratory system during eructation; 2) studies of rumen motility in dwarf cattle which showed a normal motility pattern in bulldog dwarfs but a random, continuous motility in the single achondroplastic dwarf studied. The random pattern could be made more nearly normal by treating the animal with choline-d-pantothenate or large doses of Lentin; 3)various alkaloids were assayed for emetic potency and potential usefulness in the treatment of bloat. Dry bloat in dwarf cattle was successfully and rapidly relieved by intravenous or intramuscular injection of three different alkaloids, and 4) a study of the possible role of rumen bacterial endotoxin in the etiology and pathogenesis of diet-induced diseases in ruminants was begun. (Ames, Iowa) (ADP a7-27)

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AREA NO. 8 - FOOT-AND-MOUTH AND OTHER EXOTIC INFECTIOUS DISEASES OF CATTLE

Problem. The Congress in 1948 authorized establishment of a laboratory in the United States for research on foot-and-mouth and other exotic animal diseases. The law required that the laboratory and related facilities for research and study be located on a coastal island separated from the mainland by deep, navigable waters. Plum Island was selected as the site for the laboratory on July 28, 1952. The Plum Island Animal Disease Laboratory as a U. S. Department of Agriculture venture came into existence on July 1, 1954, and since that time this laboratory has been responsible for protecting the nation's livestock industry against animal diseases of foreign origin. Foot-and-mouth disease has visited the United States on 9 occasions and each time has been eradicated. The last outbreak of foot-and-mouth disease was in 1929. Contagious bovine pleuropneumonia was eradicated in the 1880's and has not recurred since. Success in keeping these exotic animal diseases out of the United States has been due to a number of factors and a continuing vigilance by U. S. Department of Agriculture personnel.

The establishment of the Plum Island Animal Disease Laboratory and its continuing research program on exotic animal diseases has provided a laboratory in the United States where research on animal disease foreign to our soils is carried out. As new information is developed at the laboratory, it is made available to those agencies in the Department responsible for keeping out livestock animal diseases which do not occur in this country. Foot-and-mouth disease is capable of reducing our overall productivity by 25% in areas where it might become established. The disease exists in all large land areas of the world with the exception of Central and North America, Australia, and New Zealand.

Rinderpest, a disease of cattle, continues to be a serious disease problem in Africa and Asia. This disease is capable of killing 90% or more of the cattle exposed to it. Other diseases for which the laboratory is responsible include contagious bovine pleuropneumonia, Rift Valley fever, East Coast fever, and lumpy skin disease. All of these diseases continue to cause severe losses in other parts of the world. The possibilities of entry of these diseases in the United States continues, primarily because of the progressively increasing scope, speed, and extent of modern international transportation. Information developed at the Plum Island Animal Disease Laboratory is applied to the protection of the nation's livestock against foreign animal diseases.

The research continues to develop and maintain a competence for diagnosis of exotic animal diseases. Fundamental research is being conducted on biological, chemical, and physical properties of the infective agents that may be useful in prevention, control, and eradication of these diseases.

USDA AND COOPERATIVE PROGRAM

The Department at its Plum Island Animal Disease Laboratory has a continuing long-term program involving veterinarians, biochemists, biophysicists, microbiologists, and pathologists engaged in basic and applied research in this problem area. All of this research is conducted at the Plum Island Animal Disease Laboratory, Greenport, New York, except for supplemental field studies on foot-and-mouth disease vaccines which is conducted cooperatively in the Netherlands. The Department is also engaged in research under terms of an Interagency Agreement with the Assistance In Development Program, U. S. State Department, in Kenya, on contagious bovine pleuropneumonia.

The Federal scientific effort devoted to research in this area conducted solely at the Plum Island Animal Disease Laboratory, totals 28.5 professional man-years. This effort is divided among sub-headings as follows:

Histopathology -- foot-and-mouth and other exotic diseases 1.0

Fluorescent antibody technique to locate viruses 1.0

Studies on foot-and-mouth disease virus 2.0

Determine mechanism of antibody formation 0.5

Immune response of cattle to types and sub-types of foot-and-mouth disease virus 1.0

Quantity production of foot-and-mouth disease virus 2.0

Microcinematography of cellular reaction of infected cells 0.5

Establishment and characterization of cell lines and cell strains 1.0

Mechanism of the interaction between foot-and-mouth disease virus molecules and host cells 2.0

Genetic biochemistry of foot-and-mouth disease virus 1.0

Effects of chemical and physical environment on foot-and-mouth disease virus 1.0

Bulk Freeze Drying of foot-and-mouth disease virus vaccine and antiserum 1.0

Investigations of Rinderpest in Cattle 2.5

Survival and Transmission of Foot-and-Mouth Disease Virus in Semen 1.0

Identification, purification and chemical and physical characterization of foot-and-mouth disease virus and other exotic animal viruses 2.0

Immuno-chemical investigations of foot-and-mouth disease virus 1.0

Attenuation of representative types of foot-and-mouth disease virus 1.0

Survival and inactivation of foot-and-mouth disease virus in meat and meat by-products 1.0

Biological mechanism of natural resistance and susceptibility to foot-mouth disease virus 1.0

Biological alteration of foot-and-mouth disease virus from continual residence in cell cultures 1.0

Morphological aspects of virus-cell relationships 1.0

Diagnostic and immunizing procedures for contagious bovine pleuropneumonia 3.0

Work was continued under a PL 480 grant to the Instituto Biologica, Sao Paulo, Brazil for a 5-year study of tissue culture of indigenous strains of foot-and-mouth disease virus, and experimental field vaccination.

Under a PL 480 grant to the Ministry of Agriculture, Laboratories of Foot- and Mouth Disease and Tissue Culture, Etlik, Turkey, research is under way on "Studies of Various Indigenous Types of Foot-and-Mouth Disease Virus, and the Production of a Vaccine for the Control of Foot-and-Mouth Disease in Turkey."

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Histopathology -- Foot-and-Mouth and Other Exotic Diseases

It was demonstrated that the initial mucosal and epithelial lesions of both foot-and-mouth disease and vesicular stomatitis consist of circumscribed, degenerated areas of epithelial tissue. The characteristic vesicles develop from these initial lesions; but vesiculation may not and does not always occur. This knowledge is useful in clinical recognition of the two diseases.

A histopathological survey of cattle received at Plum Island during a period of one year revealed a high incidence of sub-clinical (renal) leptospirosis. Clinical recognition of the disease had never been made on the farm where the cattle were raised. The combined microscopical and serological data indicated the probability of infection with more than one serotype. Information obtained was useful in developing concepts of the character of the disease in cattle.

Microscopic pathology of foot-and-mouth disease in pregnant and lactating mice was described.

Lesions in guinea pigs resulting from estrogen contamination of pelleted feed were described. (ADP a8-1(Rev.)

B. Fluorescent Antibody Technique to Locate Viruses

A fluorescent antibody technique for antigens and/or antibodies related to foot-and-mouth disease was developed. Commercial reagents (except the immune sera) were used. Serums from cattle infected with any of the seven types of foot-and-mouth disease (FMD) consistently gave positive or (in only two instances) suspicious fluorescent antibody reactions. Serums from cattle with a lesser degree of immunity than that conferred by frank infection with virulent virus associated with lesion development, gave negative fluorescent antibody reactions. The technique appears to be useful for detecting animals convalescent from foot-and-mouth disease (from one week to as long as 2 years following infection), but did not distinguish between types of infection. (ADP a8-2(Rev.)

C. Studies on Foot-and-Mouth Disease Virus

Foot-and-mouth disease virus (FMDV), type A, strain 119, was produced in baby hamster kidney cell cultures and treated with acetylethyleneimine (AEI). The AEI-treated preparation was used to prepare a colloidal aluminum gel (Alhydrogel) vaccine and an oil emulsified vaccine. In the animals vaccinated with oil emulsion vaccine, the neutralizing and complement-fixing antibodies were maintained at levels 30 to 200 times greater than in the animals that received the Alhydrogel vaccine and similarly to that found in animals infected with FMDV. The 19S type antibody was difficult to demonstrate in both groups and did not approach the high level produced in animals undergoing actual infection.

The oil emulsified vaccine was superior to the Alhydrogel vaccine in terms of inducing higher levels of antibody which persisted for a relatively longer period of time.

An understanding of the degree and duration of immunity conferred by FMDV as well as a knowledge of practical methods for evaluating immunity is essential to properly plan and implement field vaccination programs. Investigations of this type require the use of large numbers of cattle over long periods of time and can best be carried out in an area where FMD vaccination is routinely practised. Such studies are being pursued in Holland in cooperation with the Netherlands Ministry of Agriculture. A number of herds consisting of approximately 400 cattle vaccinated and held under field conditions, are included in this study. Vaccinated cattle presented for slaughter at the Amsterdam abattoir are also available.

Serum antibody levels against types O and A FMDV remained high for over two years in most cattle which had received two or more annual vaccinations. An average of 80% of the animals which had experienced three or more annual field vaccinations showed resistance 16-48 months later when exposed to virulent FMDV. In general, a good correlation was observed between serum antibody level and resistance to infection, however, indications are that this relationship may vary somewhat depending on the interval of time elapsing between vaccination and exposure to the virus. (ADP a8-8(Rev.)

D. Determine Mechanism of Antibody Formation

The serologic, physical and chemical characteristics of antibodies produced by guinea pigs and cattle following infection with FMDV or inoculated with inactivated virus preparations, were investigated. The first appearing antibodies were macroglobulins having several different characteristics than the late appearing antibody. The time course of appearance of these two antibody types in the intact animal were studied to provide a better basis for subsequent cellular level experimentation. (ADP a8-10(Rev.)

E. Immune Response of Cattle to Types and Sub-Types of Foot-and-Mouth Disease Virus

Antibody formed in guinea pigs against the noninfective component of FMDV is specific for the noninfectious component, possesses no detectable virus neutralizing activity, and with the limited number of strains tested, appears to be type specific.

Assays of virus neutralizing antibody by tissue culture methods consistently resulted in lower values than those obtained when suckling mice were used as the assay system. Factors influencing this difference were the time and temperature of incubation of the antibody-virus mixture and the presence of a "persistent" fraction. (ADP a8-11(Rev.)

F. Quantity Production of Foot-and-Mouth Disease Virus

A simplified method for preparing bovine calf kidney cells for growth on glass has been developed. Yields of 60 ml. of packed cells are routinely obtained from 100 Gm. of cortical tissue. One thousand or more plaque-forming units (PFU) of FMDV per cell were obtained from cell cultures prepared by this method for growth and assay of the virus.

Although dispersed cells were centrifuged to remove trypsin before preparing cultures, satisfactory cultures were prepared from dispersed cells which had not been centrifuged.

Application of information developed during the year on the effect of various chemical and physical factors on cell susceptibility has resulted in preparation of primary bovine calf kidney cells with increased susceptibility to infection with FMDV. These cells were used for plaque assay

of all seven types of FMDV isolated directly from animals. Plaque assay titers were similar to those obtained in adult steers and suckling mice, demonstrating the utility of these cells for use in detection and assay of field samples of FMDV. Increased yields of FMDV from early passage in these cells reduces or eliminates the necessity of adapting FMDV to tissue culture. This finding may have important application to vaccine production.

For a 7-month period considerable effort was diverted to experiments on detection and assay of virus from lymph nodes of cattle inoculated in Argentina with FMDV. (ADP a8-12(Rev.)

G. Microcinematography of Cellular Reaction of Infected Cells

Development of a microcinematographic technique that is practical for use under animal disease quarantine conditions was accomplished at PIADL.

First indications of culture survival after infection with FMDV were observed in connection with work on this project. This information initiated a line of investigation carried on under Line Project ADP a8-30.

Observations were recorded on the cytopathic effect of FMDV and rinderpest on a variety of cultured cells. (ADP a8-13(Rev.)

H. Establishment and Characterization of Cell Lines and Cell Strains

A lamb testis cell line developed at PIADL was used in determining the neutralizing activity of serums containing antibody against mucosal disease virus. Cells of the lamb testis line have withstood freezing and storage at about -70C for three and one-half years. (ADP a8-14(Rev.)

I. Immuno-Chemical Investigations of Foot-and-Mouth Disease

The physical-chemical characteristics of antibodies produced by guinea pigs and cattle in response to infection with FMDV were investigated. Animals responded with the early appearance of a macroglobulin type antibody (19S) that disappeared by about the 30th day following infection. Within a few days following the appearance of the macroglobulin antibody (19S), antibody of a smaller size (7S) was demonstrated and this antibody persisted at high levels over an extended period of time. Various other serologic, physical and chemical differences were also found for these two types of antibody. Cattle immunized with inactivated virus preparations showed some qualitative and quantitative differences in the time course of appearance of these antibodies when compared to infected animals. Inactivated virus emulsified in oil induced higher antibody levels that persisted for a longer time than when the inactivated virus was adsorbed to aluminum hydroxide gel.
(ADP a8-26)

J. Attenuation of Representative Types of Foot-and-Mouth Disease Virus

Concentrations of glycidaldehyde (GDA) as low as 0.005%, inactivated high titered FMDV of guinea pig vesicular fluid origin. A 0.05% concentration was effective in less than 30 minutes. No GDA-resistant virus particles were detected during the inactivation studies. Inactivation rate was directly related to the ambient temperature up to 42°C and virtually disappeared at 5°C.

Glycidaldehyde compared very favorably with acetylethyleneimine as an inactivant for FMDV.

The neutralization of GDA by sodium thiosulfate is not instantaneous and concentrations as high as 10% failed to neutralize the virucidal activity of GDA.

The reactivity of GDA-treated virus with specific immune serum was essentially the same as for control preparations. CF titers as high as 1:240 were consistent in both GDA-treated and untreated antigen.

The precipitin pattern, using the agar-gel technique, did not indicate virus breakdown. Precipitin antibody was detected 7 days postinoculation from a single injection of GDA-inactivated virus. (ADP a8-27)

K. Survival and Inactivation of Foot-and-Mouth Disease Virus in Meat and Meat By-Products

A total of 42 Argentine cattle, repeatedly vaccinated against FMD with trivalent vaccines (Types A, O and C), were selected for this experiment and tested in 3 groups. Each group of 14 vaccinated and 5 unvaccinated (control) mature cattle was infected with one of the 3 types (either A, O or C) FMDV. They were slaughtered 30 to 34 hours after infection. One lymph node was removed from one hind leg of each steer at slaughter. The corresponding lymph node from the other leg was obtained after ripening (chilling) the whole carcasses for 3 days. Several other lymph nodes were packed between meat chunks in wet salt-cure in barrels.

All lymph node samples and barrels of cured meat were shipped under strict safety precautions to the Plum Island Animal Disease Laboratory, U.S.A., and tested for the presence of virus using the most sensitive methods known. FMDV was detected in all fresh and all but one ripened lymph node sample from 15 unvaccinated (control) cattle. Virus also was found in 4 of 15 salt-cured samples held at 4C, 38-39 days. After infection, 2 of 42 vaccinated Argentine cattle developed tongue lesions containing virulent virus. Foot-and mouth disease virus was isolated from a lymph node in 1 of these 42 unvaccinated cattle.

In view of these results, it was concluded that FMDV may be present in the lymphatic system of vaccinated, and subsequently infected cattle. Presently

available vaccination methods do not prevent the dissemination of FMDV through meat.

Using 7 known types of FMDV, A, O, C, SAT-1, SAT-2, SAT-3, and Asia 1, it was shown that bovine lymph nodes contain virus as early as 12 hours and as long as 15 days after inoculation. While considerable amounts of FMDV may be present in lymph nodes, it might be difficult to diagnose the disease by routine inspection procedures at the preclinical and convalescent stages of infection. Cattle slaughtered during the course of inapparent infection may propagate FMDV through animal products.

Foot-and-mouth disease virus was detected in joints of infected cattle and survived in synovial fluid of infected carcasses for 19 days when stored at 4C. Virus remained infectious for several weeks in joints stored successively at chilling, freezing, and thawing temperatures.

Foot-and-mouth disease virus was remarkably stable in blood and infected or contaminated animal tissues which had been spread on materials used to package meat (wood, paper, metal). In several tests, the virus survived 48 days in blood spread on a can and stored at 4C. These preliminary results indicated that meat shipping containers may play a significant role in disseminating FMDV. (ADP a8-28)

L. Biological Mechanism of Natural Resistance and Susceptibility to Foot-and-Mouth Disease Virus

Infant mice are highly susceptible to FMDV but develop a pronounced resistance as they mature. However, during the period of late pregnancy to about two weeks postpartum, 50-70% of female mice will succumb to FMDV. Factors which might be related to the mechanisms of these responses have been investigated:

1) Suspensions of minced kidneys from individual 1-week-old mice, which are uniformly susceptible to FMDV, produced virus quickly and to high titers with only slight variation between preparations. In cells from less susceptible 5-week-old mice, however, there was considerable variation between preparations in time when multiplication began, time of peak titer, and amount of virus produced.

2) Mother mice with litters reacted with much less sensitivity after passive transfer of serum from mice sensitized with bovine serum than did similar mothers following removal of litters or nonmated controls. Similarly, serum from sensitized mother mice with litters produced less sensitivity in nonmated female mice than did sera from mother mice without litters or from nonmated control mice.

3) After being subcultured eight times cells originating from calf kidneys were less susceptible to FMDV than cells from the primary culture. Experiments indicate that selection of resistant cells occurs during subcultivation and that many serial passages of FMDV in primary cells results in the selection of virus with more virulence for the subcultured cells.

(ADP a8-29)

M. Biological Alterations of Foot-and-Mouth Disease Virus from Continual Residence in Cell Cultures

Type C_3 Rio foot-and-mouth disease virus and several line of type A-119 have been established in chronic residence on cultured bovine kidney cells. In all instances, a reduction in virulence has been effected. Total virus in work harvests from calf kidney cultures ranges from $10^{6.5}$ to $10^{8.6}$ $TCID_{50}$. The amount of virulent virus in the various harvests (as determined by intralingual inoculations of cattle) ranges from 10^2 bovine ID_{50} to 10^5 bovine ID_{50} depending on time in chronic residence and degree of modification. Indications are that the loss in virulence results from virus selection rather than a mutation or genetic change.

Conventional rapid serial passage of one line of modified virus 14 times in calf kidney cultures resulted in an increase of one log of residual virulent virus in the final harvest ($10^{2.0}$ to $10^{3.1}$ ID_{50}) and partial restoration of the plaque forming ability ($10^{5.7}$ as compared with $10^{7.5}$ $TCID_{50}$). However the plaques were very small compared with average plaque produced by normal virus.

Virus populations with reduced virulence for cattle also had reduced virulence, but significant antigenicity for sheep.

Resistant cell lines developed from cultures cured of chronic infection may have value for use in more rapid selection of avirulent virus populations.

(ADP a8-30)

N. Morphological Aspects of Virus-Cell Relationships

Work was begun on the initial phase of the project, namely, development of primary or permanent cell lines which would react slowly to FMDV, or replicate the virus without destruction of the cells. Cultures of this type would be advantageous to morphological studies of virus-cell relationships.

Four primary cell lines were developed: two of bovine origin, one of swine origin and one of canine origin. All have low susceptibility to FMDV except the one of canine origin which appears to be refractory. Two of the lines were developed after suppression of the highly susceptible cells in the original cultures by viral action (in connection with ADP a8-30). None of the cultures have been carried long enough to determine if they have the indefinite growth potential of permanent cell lines. The cultures are being

observed for degree of susceptibility, conditions related to spontaneous cure of infection, and susceptibility to reinfection after cure.
(ADP a8-31)

O. Investigations of Rinderpest in Cattle

Rinderpest virus, strain, Kabete O, was attenuated in tissue cultures. An avirulent virus population was segregated using the terminal dilution technique. The virus has been studied in laboratory tests and has shown promise as a vaccine in cattle and sheep. It has not shown properties to revert to full virulence in either species. The thermal and hydrogen-ion properties of rinderpest virus have been determined using cell culture techniques.

A sample of the rinderpest vaccine developed at PIADL has been made available to a Food and Agriculture Organization Laboratory of the United Nations located in Cairo, Egypt. The vaccine is being studied in that country for its usefulness in protecting animals against exposure to rinderpest.
(ADP a8-23)

P. Studies on Foot-and-Mouth Disease Virus (PL 480 Project)

Research is being conducted on FMDV at the Instituto Biologica, Sao Paulo, Brazil. At this Institute the investigators are serially passaging certain types of FMDV in a variety of types of tissue cultures. Following passage, the viruses are examined to determine the immunizing properties and according to the results recently received from that Institute, there is evidence that at least some of the viruses are becoming somewhat attenuated for cattle. The workers at this Institute have shown that tissue cultures are exceptionally valuable in diagnosis of samples submitted from the field. Using tissue cultures as an indicator medium and saliva from affected animals as a test sample, positive identification of FMDV has resulted in a large percentage of the samples so examined. The workers at this Institute have also developed a cell line from the kidneys of swine which is useful in primary isolation of virus from samples submitted from the field. The cell line which has been developed by workers at this Institute multiplies rapidly, may be easily subcultured, and is sensitive to all of the types of FMDV against which it has been tested. It may well be useful for production of virus for commercial fabrication of FMD vaccine.
(S3-ADP-2)

Q. Studies on Various Indigenous Types of Foot-and-Mouth Disease Virus, and the Production of a Vaccine for the Control of FMD in Turkey (PL480 Proj)

This investigation is in progress under a PL 480 Grant to The Ministry of Agriculture, Laboratories of Foot-and-Mouth Disease and Tissue Culture, Etlik, Turkey. The work is still in the preliminary stage, since the grant was made during the reporting period. (A22-ADP-8)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Rinderpest

DeBoer, C. J., and Barber, T. L. 1963. Studies with Tissue Culture-Modified Rinderpest Virus as an Immunizing Agent. Proc. XVII World Vet. Cong., 1:611-613.

DeBoer, C.J., and Barber, T. L. 1964. Segregation of an Avirulent Variant of Rinderpest Virus by the Terminal Dilution Technique in Tissue Culture. J. Immunol. 92.

DeBoer, C. J., and Barber, T. L. 1964. Segregation of an Avirulent Variant of Rinderpest Virus by the Limiting Dilution Technique in Tissue Culture. Repr. Fed. Proc., V:2:22.

Foot-and-Mouth Disease - Microbiological Investigations

Cottral, G. E., Gailiunas, P., and Campion, R. L. 1963. Detection of Foot-and-Mouth Disease Virus in Lymph Nodes of Cattle Throughout Course of Infection. 67th Ann. Meet. U.S. Livestock Sanit. Assn., pp. 463

Foot-and-Mouth Disease - Immunological Investigations

Graves, John H. 1963. Formaldehyde Inactivation of Foot-and-Mouth Disease Virus in Vaccine Preparations. Amer. J. Vet. Res., 24:103:1131-1136.

Graves, John H. 1963. Transfer of Neutralizing Antibody by Colostrum to Calves born of Foot-and-Mouth Disease Vaccinated Dams. J. Immuno. 91:2: 251-256.

Graves, John H., Cowan, K. M., and Trautman, R. 1964. Characterization of Antibodies produced by Guinea Pigs Inoculated with Inactivated Foot-and-Mouth Disease Antigen. J. Immuno. 92:4:501-506.

Foot-and-Mouth Disease - Histopathological Investigations

Seibold, H. R. 1963. A Revised Concept of the Lingual Lesions in Cattle with Foot-and-Mouth Disease. Amer. J. Vet. Res., 24:1123-1130.

Foot-and-Mouth Disease - Cytological Investigations

Seibold, H. R., Cottral, G. E., and Patty, R. E. 1964. Apparent Modification of Foot-and-Mouth Disease Virus after Prolonged Residence in Surviving Cells. Amer. J. Vet. Res., 25:806-814.

Foot-and-Mouth Disease - General

Campbell, C. H. 1963. Influence of tissue culture passage on virulence of foot-and-mouth disease virus for mother mice. J. Bacteriol. 86:593-597.

Campbell, C. H. 1964. Relation of physiologic conditions to variations in susceptibility of mother mice to foot-and-mouth disease virus. J. Immunol. 92:6:858-863.

AREA No. 9 FOOT-AND-MOUTH AND OTHER EXOTIC DISEASES OF SWINE

Problem. Foreign diseases, such as foot-and-mouth disease, African swine fever, and Teschen disease, that occur elsewhere in the world, constitute calculable potential threats to the swine industry of the United States. Foot-and-mouth disease is of particular importance because the disease frequently occurs primarily in swine from which it spreads to other susceptible species, such as cattle and other ruminants. African swine fever, which until recently was confined to wild and domestic pigs in Africa, has spread to Portugal, Spain, and France. The disease is of special concern because of its resemblance to hog cholera, with which it may be confused. Moreover, mortality from the disease approaches 100 per cent, and there is no specific preventive vaccine. Teschen disease, which causes widespread inapparent infections and occasional involvement of the central nervous system, is another of the foreign diseases to be guarded against. A disease indistinguishable from Teschen disease has appeared in England in recent years. Despite all precautions, any of these diseases may occur in the United States, as likely as not through the medium of modern, rapid international transportation. The Plum Island Animal Disease Laboratory is engaged in studies of foreign diseases of swine for the purpose of developing information for increased protection of the Nation's swine industry.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving veterinarians, biochemists, microbiologists, and pathologists, engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 6.6 professional man years. This effort is divided among sub-headings as follows:

Foot-and-Mouth Disease of Swine 1.0 at the Plum Island Animal Disease Laboratory, Plum Island, New York.

African Swine Fever 4.6 at the Plum Island Animal Disease Laboratory in cooperation with the East African Veterinary Research Organization, Muguga, Kenya, and in connection with a PL 480 project in Madrid, Spain, where the equivalent of $97,550 has been made available to the Spanish Ministry of Agriculture over a 3-year period.

Rinderpest in pigs 1.0 at the Plum Island Animal Disease Laboratory, Plum Island, New York.

PROGRAM OF STATE EXPERIMENT STATIONS

Experimentation with the virus of foot-and-mouth disease in the United States essentially is prohibited by law except at the Plum Island Animal Disease Laboratory. Experimentations with the causative agents of other communicable foreign, or exotic diseases of swine in the United States is similarly prohibited generally by federal regulations. Consequently, the State Experiment Stations are not working with diseases in this category.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Foot-and-Mouth Disease of Swine

Foot-and-mouth disease in swine might well go unnoticed in those areas of the United States where vesicular stomatitis exists. Both viruses readily infect swine and clinically it is impossible to distinguish the infection. Foot-and-mouth disease virus, however, is more infective and generally causes a higher mortality than does the vesicular stomatitis virus. The problem is to conduct basic research to develop better means of diagnosing, control, and eradication of foot-and-mouth disease in swine. In those countries where foot-and-mouth disease exists, swine are the next most frequently affected animal, cattle being the principal species. Little is known concerning the infection in swine, nor is there a vaccine for use in this species. Vaccines used in cattle, while not perfect, do produce immunity after repeated vaccinations. This is not true in swine, thus vaccination is not widely practiced as a control measure against the disease in this species.

Studies have been commenced in swine using foot-and-mouth disease virus (FMDV), type C-3, CANEFA (designates a strain isolated from an outbreak in Argentina). Virus appears as early as 24 hours and persists for approximately 96 hours. Antibody was detected as early as 96 hours both by the variable serum-constant virus neutralization test in suckling mice and the Ouchterlony agar gel precipitin test. The early antibody as indicated by density gradient ultracentrifugation examination was of the 19S type. By the 7th day, both the 19S and 7S were present. The antibody level appeared to reach a peak by the 7th or 8th day. The virus modified by residence in primary bovine kidney cells for 182 days would appear to be insufficiently modified for other than experimental study. (ADP a9-1)

B. African Swine Fever

Until approximately 6 years ago, African swine fever (ASF) had never been known to occur outside Africa. Following introduction into Portugal in 1958, it spread to Spain. Efforts to eradicate the disease in these countries have not been successful. This is in spite of the use of attenuated vaccines. Early in 1964, the disease spread to southern France and by May had spread as far north as Brittany. Due to the incidence of hog cholera (European swine fever) in this part of France, difficulties are being

experienced in diagnosing ASF. While there is a laboratory test for diagnosis of ASF, difficulties are experienced in France in the use of the hemadsorption test for distinguishing ASF from hog cholera.

Basic and applied research is conducted by bacteriologists, chemists, and virologists at the Plum Island Animal Disease Laboratory, and at the East African Veterinary Research Organization. In East Africa, the research is being conducted under terms of a cooperative agreement with the East African Veterinary Research Organization, Muguga, Kenya, East Africa. Research is being conducted to develop information concerning the number and types of virus which may exist. In the approximately 8 years, during which USDA scientists have been working on the disease, 38 samples of virus have been collected and workers are in the process of comparing these to determine whether more than one immunologic type of virus exists.

During the past year samples of the virus responsible for three new outbreaks (Rhodesia, France, and a new area of Kenya) have been obtained. Of these specimens, 15 are from domestic pigs, 17 from wart hogs, 5 from bush pigs, and 1 from porcupine. During the past year, tissues from more than 300 hippopatomi have been examined and all have been shown to be free from ASF viruses. It was previously thought that this animal, along with the hyena, porcupine, bush pig, and wart hog, may be implicated in the spread of the disease. Following examination of the 300 specimens, it has been concluded that the hippopatomus does not play a role in the epizootiology of African Swine Fever.

Two strains of pig kidney cells and a strain of baby hamster kidney cells, have been found satisfactory for producing quantities of African Swine Fever virus. (ADP a9-2)

Under the terms of a PL 480 agreement, research is being conducted at the Servicio de Patologia, Patronata de Biologia Animal, Embajadores, Madrid, Spain, on rapid and accurate diagnostic methods for African Swine fever. USDA scientists, working on ASF in Africa, developed a laboratory test for the diagnosis of ASF. This is based on the adsorption of red cells onto cultures of buffy coat cells. Only those cells which are infected with ASF virus will adsorb red blood cells. The occurrence of ASF in Spain, and the need to conduct diagnosis on samples suspected of being ASF, provided an opportunity to study this method of diagnosis under actual conditions. The Spanish work has shown the test to be specific for ASF. They have published variously on their application of the hemadsorption test for diagnosis of ASF. For the most part, these publications have appeared in Spanish veterinary journals and in publication media of the Office of International Epizootics, Paris, France. (Spain) (E25-ADP-4)

C. Rinderpest in Pigs

A strain of rinderpest virus (Pendik isolate) which caused death in cattle was serially passaged 15 times in pigs. The clinical response of pigs included a rise in temperature and a reduction in white cell count which was statistically significant (P.01) at passage levels 0 (bovine origin virus), 5, 10, and 15. The virus did not increase in virulence for the pig as indicated by the absence of mortality. The virus retained its pathogenicity for cattle during the serial passage in swine. (ADP a9-3)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

African Swine Fever

DeLay, P. D., and Carbrey, E. A. 1963. Experimentally induced hog cholera in pigs immunized with African swine fever. Proc. U. S. Livestock San. Assn. pp. 170-176

Rinderpest in Swine

Barber, T. L., and Heuschele, W. P. 1964. Experimental passage of rinderpest virus in North American pigs. Bull. Epiz. Dis. Africa. Oct.

AREA NO. 10 - FOOT-AND-MOUTH AND OTHER EXOTIC DISEASES OF SHEEP

Problem. For the early detection of any outbreak of foot-and-mouth disease, comprehensive information regarding its effect on all susceptible species is necessary. The effect of foot-and-mouth disease (FMD) on cattle and swine has been, and is being investigated, however, little information is available pertaining to the disease in sheep. Sheep infected with FMD could serve as a source of infection and initiate the spread of the disease. Although primary research emphasis on exotic diseases of sheep at the Plum Island Animal Disease Laboratory is on FMD because of its great economic importance, other exotic diseases of sheep, such as rinderpest, sheep pox, louping ill, Nairobi sheep disease, and Rift Valley fever, are of concern to the Plum Island Laboratory because techniques and materials may be needed for diagnosis, control, and eradication on short notice and unexpectedly. Such diseases, if introduced into this country, could result in high death tolls or cause serious economic losses among susceptible sheep and other livestock. The problem is one of development of basic information applicable to protection of the nation's sheep from foreign animal diseases; development and maintenance of competence in diagnosis of these diseases, and fundamental research on the biological, chemical, and physical properties of the infectious agents that may be useful in prevention, control, and eradication of these diseases.

USDA AND COOPERATIVE PROGRAM

The Department has recently activated a continuing and long-term program involving veterinarians, biochemists, microbiologists, and pathologists, engaged in basic and applied research in some of the problems in this area.

The Federal scientific effort devoted to research in this area totals 2 professional man-years. This effort is divided among sub-headings as follows:

Foot-and-Mouth Disease of Sheep, 1.0 at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York.

Rinderpest in Sheep, 1.0 at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York.

Sheep Pox, Public Law 480 funds have been made available to the Turkish Ministry of Agriculture for a 2-year study of vaccines against sheep pox prepared from tissue culture propagated virus. The Madras Veterinary College, Madras, India, has also received PL 480 funds to conduct research on an efficient vaccine for protecting sheep against sheep pox. Sheep pox is indigenous in Turkey and India.

PROGRAM OF STATE EXPERIMENT STATIONS

None.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Foot-and-Mouth Disease in Sheep

Virus-neutralizing, complement-fixing and precipitating antibodies were detected in the serums of sheep following infection with foot-and-mouth disease virus (FMDV). Investigations have been conducted on the persistence of these antibodies. Virus-neutralizing antibodies were present 518 days postinoculation; precipitating antibodies 560 days postinoculation. Testing for complement-fixing antibody beyond 350 days has not been completed. The persistence of antibodies in sheep serums following infection with FMDV and the fact that neutralizing and precipitating antibodies may be readily detected is significant from a regulatory standpoint. (ADP all-1)

B. Rinderpest in Sheep

Sheep were experimentally infected with a bovine-lethal strain of rinderpest virus. The clinical response consisted of an elevated temperature during the third through the seventh day after inoculation and a reduced total white blood cell count beginning on the second, and persisting through the twelfth day after inoculation. Signs of illness were not obvious and gross lesions were not found. (ADP all-2)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Foot-and-Mouth Disease

Dellers, Robert W. and Hyde, John L. 1964. Response of Sheep to Experimental Infection with Foot-and-Mouth Disease Virus. Amer. Jour. Vet. Res. 25(105):469-473 :March .

Rinderpest

Barber, T. L. and Heuschele, W. P. 1963. Experimental Rinderpest in Sheep. Proc. 67th Ann. Meet. USLSA. 155-162

AREA NO. 11 - PARASITES AND PARASITIC DISEASES OF CATTLE

Problem. The cost of parasitic diseases to the cattle industry of the United States is estimated to be in excess of $400 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy cattle, insure adequate supplies of parasite-free beef for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a more prosperous agriculture and the national economy.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, parasitologists, pathologists and veterinarians engaged in both basic and applied studies directed to the development of measures for the solution to the high and extremely costly incidence of parasitism in cattle. Research is being conducted on parasitic diseases at the following designated locations.

The Federal scientific effort devoted to research in this area totals 21.5 professional man-years. This effort is divided among subheadings as follows:

Ecological Factors Influencing Gastro-Intestinal Nematodes of Cattle 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Effect of Pasture Mixtures and Pasture Management on Control of Internal Parasites 1.5 at the Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Acquisition and Effects of Roundworm Parasites of Cattle as Influenced by Diet 1.5 at the Animal Disease and Parasite Research Division, Beltsville Parasitological Laboratory, Beltsville, Maryland.

Cultural Characteristics and Artificial Propagation of Protozoan Parasites 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Host-Parasite Relationship of Coccidial Parasites of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Ecology and Immunology of the Cattle Lungworm, Dictyocaulus viviparus 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle, Including Anthelmintic Treatment 2.0 at the University of California, Davis, under a cooperative agreement with the ARS-USDA.

Investigations of Trichomonad Parasites 1.0 at the Animal Disease and Parasite Research Division Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Utah Agricultural Experiment Station, Logan, Utah.

Host-Parasite Relationship of Intestinal Worms, Cooperia spp. in Cattle 2.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Epizootiological and Ecological Investigations of the Internal Parasites of Grazing Cattle 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Etiology and Immune Response of Cattle to Winter Coccidiosis 1.0 at the Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Montana Agricultural Experiment Station, Bozeman.

Anaplasmosis of Cattle 4.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and through a memorandum of understanding and other agreements in cooperation with the State Experiment Stations in California, Illinois, Louisiana, Nevada, and State Veterinarian of Tennessee, the USDA Entomology Research Station, Kerrville, Texas, and the Delta Branch Experiment Station, Stoneville, Mississippi.

Interrelationships of Diet and Parasitic Infection in the Production of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Histochemistry of Gastro-Intestinal Nematodes of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Parasites of Cattle with emphasis on Stephanofilarial Species 1.0 at the Animal Disease and Parasite Research Division Regional Animal Disease Laboratory, University Park, New Mexico.

Environmental Factors Influencing Parasites and Parasitic Diseases of Economical Importance in Ruminants (Cattle, Sheep, and Alpacas)(PL-480 Peru)

Investigations on Anaplasmosis, Piroplasmosis and Babesiallosis of Cattle are under way through a PL 480 Grant at the School of Veterinary, Montevideo, Uruguay (PL 480 Uruguay)

PROGRAM OF STATE EXPERIMENT STATIONS

Twelve Western States and the Department are cooperating in regional research on internal parasitological problems of cattle (W-35). Informal coordination is maintained with States in the southern region also working on this subject. New and improved methods for diagnosing nematode parasitic diseases are being developed and relationships between types of pasture forages and degree of parasitism are being established. Biological and chemical controls are under evaluation. The effects which promising anthelmintics have upon weight gains and feed efficiency of parasitized cattle are being measured.

Basic studies are seeking to establish how nematodes damage the host animal, interfere with nutrition and bring about disease. Studies of biochemical systems involved in parasite metabolism and the effect of anthelmintics on these systems are providing key information necessary in developing improved therapeutic measures.

Other studies are aimed at reducing exposure to cattle parasites through the development of systems for managing grazing and feeding procedures. Factors which favor over-winter survival of infective parasite larvae are being determined and micro-climatic conditions conducive to larval infectivity are being established. Studies at several locations are in progress on coccidiosis of cattle to determine the conditions favoring outbreaks of this disease. Factors affecting immunity to this parasite are being determined. Basic information is being sought at a number of States on the nature of Anaplasma in order to elucidate the life cycle of this parasite and provide a means for its control. Preventive immunization is under study and methods of eradication are being explored.

The total State scientific effort devoted to this research is 8.9 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Etiological Factors Influencing Gastro-Intestinal Nematodes of Cattle.

1. Investigations made at Experiment, Georgia, under the auspices of the Regional Animal Disease Laboratory at Auburn, Alabama, showed a reduction in the number of infected larvae of various cattle and sheep nematodes proportional to the increase in number of viable spores of Bacillus thuringiensis var. thuringiensis Berliner (Rohm and Hass Co., and Stauffer Chemical Co.,) incorporated in feces-worm egg cultures. Some of the same materials fed to calves and sheep proved to be toxic, without any appreciable reduction in the number of larvae obtained from fecal cultures from these hosts. (ADP bl-6(Rev.)

2. Studies have shown that a grain ration (corn) appears to have an inhibitory action on the development in the feces of the larvae of the five species of cattle nematodes (Trichostrongylus axei, T. colubriformis, Oesophagostomum radiatum, Cooperia oncophora, and C. pectinata.) Apparently, silage does not have any effect on larval development. (ADP bl-6(Rev.)

3. Sporangia of a mold, Pilobolus spp. was observed to disseminate infective larvae of nematode parasites of cattle present on the vesicle at the time the sporangia were discharged. This may constitute another way for the dissemination of nematode larvae from the fecal pad in the field to the forage to be consumed by ruminants. This report confirms earlier observations made in England. (Alabama and Georgia) (ADP bl-6(Rev.)

B. Acquisition and Effects of Roundworm Parasites of Cattle as Influenced by Diet.

At the Beltsville Parasitological Laboratory (BPL), Beltsville, Maryland, four experiments, using a total of 37 calves, were performed to determine whether feeding a normal level or a subsistence level of combinations of grain and hay for 7 weeks preinoculation of larvae would effect their resistance to infection of gastro-intestinal worms. The calves weighed from 171 to 331 lbs. at the time of inoculation. Lots 1 and 2 received 248,000 and 116,000 larvae, respectively, of the medium stomach worm. Lots 3 and 4 received a total mixture of 485,000 and 523,000 larvae of the medium stomach worm and 6 other species of gastro-intestinal parasites, respectively. Calves were necropsied 6 to 7 weeks post-inoculation.

The parasitized calves on the low feeding level were outgained by their respective controls by 12.2 lbs./100 lbs. of TDN consumed (total digestible nutrients) (Avg. of all 4 experiments). The parasitized calves on the higher feeding level were outgained by their controls by 9.2 lbs./100 lbs. of TDN consumed. The parasitized calves on the higher feeding level outgained those on the lower feeding level by 11.8 lbs./100 lbs. TDN consumed and the uninfected control calves on the higher feeding level outgained those on the lower feeding level by 11.5 lbs./100 lbs. TDN consumed.

Analysis of these results showed that moderate infections with gastrointestinal nematode parasites reduced the efficiency of feed utilization by calves more than did mild infections, but feeding level did not appear to affect the susceptibility of the calves to infection with these parasites under the conditions of the experiments. (Maryland) (ADP b1-19 R)

C. Cultural Characteristics and Artificial Propagation of Protozoan Parasites.

Experiments at the Beltsville Parasitological Laboratory on the symbiotic relationship between the protozoan parasite, Histomonas meleagridis, and the enteric (intestinal) bacterial flora of its natural hosts, chickens and turkeys, and from several small mammals, showed bacteria from the turkey were more beneficial to the parasite than were those from the chicken; those from small mammals were without benefit. This finding may provide at least a partial explanation of why this parasite is more damaging to the turkey than to the chicken.

The probiotic activity for Histomonas of turkey cecal bacteria was not destroyed by heating at a temperature of 56°C (132.8°F) for 30 minutes or at lower temperatures for as long as 60 minutes. At temperatures of 65°C (149°F) or higher the activity of the bacteria was destroyed within about 5 minutes. (Maryland) (ADP b1-22)

D. Host-Parasite Relationships of Coccidial Parasites of Cattle.

The ADP Regional Animal Disease Laboratory, Auburn, Alabama, reported that for the first time observations of a preliminary nature were made on the endogenous cycles of Eimeria cylindrica and E. canadensis in calves. Schizonts, merozoites, microgametocytes and macrogametocytes of E. cylindrica were found in fresh smears and sections of the intestines at 8 days postinoculation. In E. canadensis at 15 days postinoculation, fresh smears revealed microgametocytes and macrogametocytes in the small intestine 6 and 12 feet anterior to the cecum. (Alabama) (ADP b1-23(Rev.))

E. Ecology and Immunology of the Cattle Lungworm, Dictyocaulus viviparus.

Work conducted at the Beltsville Parasitological Laboratory showed that oral vaccination of calves with infective larvae of the equine lungworm for protection against infection with the cattle lungworm was without adverse clinical effect. The procedure showed promise of efficacy. (ADP b1-24)

F. Clinical and Physiological Aspects of Roundworm Parasitism in Cattle, Including Anthelmintic Treatment.

The School of Veterinary Medicine, University of California, Davis, under a cooperative agreement with the USDA, reported research on anthelmintic treatment with the following results:

I. ANTHELMINTIC STUDIES

A. Trial 1. Activity of Bayer S-940 and Bayer S-6658 against nematodes in sheep.

1. Bayer S-940 removed 95% and 98% of nematodes from lambs at dosages of 50 mg./kg. and 100 mg./kg., respectively.

2. Bayer S-6658 removed 92% and 98% of nematodes from lambs at dosages of 200 mg./kg. and 400 mg./kg., respectively.

B. Trial 11. Activity of Bayer 9017 and Bayer 9018 against nematodes in sheep.

1. Bayer 9017 removed 65% and 90% of nematodes from lambs at 15 mg./kg., and 30 mg./kg., respectively.

2. Bayer 9018 removed 17% and 38% of nematodes from lambs at 20 mg./kg. and 40 mg./kg., respectively.

II. PHYSIOLOGICAL STUDIES

A. Iron kinetic studies in Hereford steers during the early acute phase showed no significant alterations from the normal. This would suggest that only in the later stages of the disease are alterations to be found.

B. The biological half life of total body water in cattle suffering from acute gastrointestinal parasitism was found to be about twice that of normal animals.

C. The serum albumin/serum globulin ratio was found to change from 0.49 in severely parasitized cattle to 1.00 twenty-five days after therapy. (California) (ADP bl-25)

G. Investigations on Trichomonad Parasites.

Research workers at the ADP Regional Animal Disease Laboratory, Logan, Utah, reported that a graded series of freeze-dried organisms was inoculated intravenously into two rabbits. Both rabbits showed anaphylactoid shock after each inoculation. One rabbit died after the third inoculation, and as a result, the other rabbit was given only four inoculations. The rabbit that died had a serum agglutination titer of 160 at time of death; the other rabbit produced an excellent serum titer of 2560. Serum from this rabbit, however, with the homologous antigen produced no precipitin lines on gel diffusion plates. Serum from the rabbit that died produced one precipitin line. Due to the high agglutination titer produced in the one rabbit, it is felt that further trials are warranted using freeze-dried T. foetus organisms intravenously.

Five experiments were run using different strains of Trichomonad foetus. A series of six inoculations of live washed Trichomonads, followed by a single large inoculation one month later, produced antisera with agglutination titers up to 2560 and 10240 in the first two experiments. The second inoculation did not increase the agglutination titer but did result in stronger precipitin reactions in gel diffusion plates. However, only two and three precipitin lines were formed. The antisera were freeze-dried and rehydrated to one-fourth their original volume. Gel diffusion reactions were considerably improved, producing 4 to 6 precipitin lines consistently. This concentrated serum also responded normally to electrophoresis and can be used for microimmuno-electrophoresis.

At the Utah Agricultural Experiment Station, Logan, research was continued under a cooperative agreement with the USDA on trichomonads and related flagellates of the bovine digestive tract. In the examination of animals, infection was found as follows: a) in the cecum of 1, and in the rumen of 1 of 45 cattle; b) in the cecum of 16 of 21 calves; c) in the cecum of 7 and in the rumen of 8 of 17 sheep; d) in the cecum of 14 of 14 pigs. Twelve pigs had Trichomonas suis; 7 had T. buttrey; and 4 had Trichomitus rotunda.

A pentatrichomonad was found to have the highest incidence of any flagellate in the bovine cecum and rumen. It was easily cultivated in several different media. Two strains from the rumen had similar growth curves, but in 2 clones from the cecum and 2 strains of Pentatrichomonas hominis of human and canine origin, the growth curves were distinctly different. An organism with 4 flagella arising in pairs was found in the cecum and feces of cattle, and described as Monocercomonoides bovis n. sp. (Utah)
(ADP b1-26)

H. Host-Parasite Relationship of Intestinal Worms, Cooperia species, in Cattle.

Reported research from the ADP Regional Laboratory, Auburn, Alabama, showed that calves and lambs inoculated with infective larvae of either of the intestinal nematodes, Cooperia oncophora or C. pectinata, developed immunity to challenge inoculation with either the homologous or heterologous species. One animal that failed to develop immunity to the homologous species also lacked immunity to the heterologous species.

Calves inoculated with 300,000 Cooperia pectinata infective larvae in a single dose were killed by their infections. Two that were moribund 6 weeks after inoculation had lost 20 and 21 pounds. Calves inoculated with 10 successive daily doses of 30,000 larvae each gained an average of 7.3 pounds, while non-inoculated controls averaged a 36.7 pound gain. Calves given 42 successive daily doses of 7,140 larvae gained an average of 31.5 pounds, while controls averaged 42 pounds in one test, and 77 and 78.3 pounds, respectively, in another similar test. The results are additional evidence of the pathogenicity of C. pectinata and indicate that clinical

parasitism develops in susceptible calves as the result of acquisition of infective larvae in large numbers over short periods of time. (Auburn, Ala.) (ADP b1-27)

I. Epizootiological and Ecological Investigations of the Internal Parasites of Grazing Cattle.

The Beltsville Parasitological Laboratory research workers reported that the larvae of the gastrointestinal worms of cattle can develop at a temperature of 35°F to the second stage when they have been preconditioned for two weeks at 45°F. This is a much lower temperature than is usually thought to be conducive to the development of the gastrointestinal nematodes of cattle.

The viability of eggs of the beef tapeworm of man was reduced by 80 and 90% by exposure to 50,000 r and 100,000 r of x-irradiation, respectively. Only 0.29% of the cysts (Cysticercus bovis), which cause condemnation and retention of carcasses for bovine cysticercosis under meat inspection regulations, developed from a large dose of eggs exposed to 200,000 r of x-irradiation. (Maryland) (ADP b1-28)

J. Etiology and Immune Response of Cattle to Winter Coccidiosis.

Research was continued at the Montana Veterinary Research Laboratory, Agricultural Experiment Station, Bozeman, under a cooperative agreement with the USDA. Reported observations on 10 disease outbreaks in cattle indicated that Eimeria zurnii was the predominant organism in 5 cases, E. bovis in three, and E. canadensis and E. brasiliensis each in one case. Confirmatory diagnosis of clinical coccidiosis was made in only 4 instances in which E. zurnii occurred alone. Oocyst counts on mucosal scrapings from the lower colon of the above animals varied from 19,500 to 3,612,000 oocysts/gram.

Supernatant from a saline emulsion of colonic contents of a Hereford calf that died after showing symptoms typical of convulsive coccidiosis, was highly toxic to mice when injected intraperitoneally. This supernatant was not neutralized by Clostridium perfringens anti-sera of types A, C, or D, or by Cl. septicum antiserum. This colonic supernatant remained lethal to mice, after being stored for 6 days, when injected intraperitoneally in 0.1 ml. doses. (Montana) (ADP b1-29)

Studies were continued on Winter Coccidiosis at the ADP Regional Laboratory at Logan, Utah. Three experiments were conducted involving prolonged daily oral inoculation of Holstein-Friesian calves with sporulated oocysts of Eimeria bovis and E. zurnii. In one experiment calves were inoculated 50 days with 500 or 15,000 E. bovis oocysts by adding an aqueous suspension containing oocysts to the evening feeding of milk. Each calf was given a single challenge inoculation of 500,000 oocysts after recovery from the initial prolonged inoculations.

In a second experiment, similar in objectives, calves were fed 100 or 15,000 oocysts daily in the evening feeding of milk, or 1000 oocysts in the evening feeding of grain. A third group of calves served as controls. All calves were given a challenge inoculation of 500,000 oocysts.

The results of the first 2 experiments showed that calves ingesting the least oocysts developed less severe symptoms of coccidiosis than did those ingesting the larger number. The length of time calves were susceptible to repeated inoculations was about the same in all groups and the degree of immunity was similar, although calves undergoing infections wherein large numbers of oocysts were discharged and clinical signs were severe seemed to exhibit a somewhat stronger immunity. These results confirm those reported from one experiment in last year's report.

Ten calves were inoculated with sporulated oocysts previously exposed to radiation of 10,000 r, 50,000 r, 100,000 r, or 200,000 r in a cobalt-60 source. Calves receiving oocysts irradiated at 10,000 r developed coccidiosis similar to control calves receiving non-irradiated oocysts. Those receiving oocysts exposed to 50,000 r exhibited mild coccidiosis. Those receiving oocysts exposed to 100,000 r or 200,000 r developed no evidence of coccidiosis and were completely susceptible to challenge with non-irradiated oocysts. It appeared that the sporulated oocysts exposed to 100,000 r and 200,000 r were killed by the radiation and were unable to elicit an immune response in the gut. The amount of radiation required to kill sporulated oocysts appears to be between 50,000 r and 100,000 r, probably about 75,000 r. (Utah) (ADP b1-29)

K. Anaplasmosis of Cattle

At the Beltsville Parasitology Laboratory, research workers reported the following findings: Serum samples from cattle in the incubative, acute, and carrier stages of bovine anaplasmosis were tested by the agar gel precipitin technique and the complement-fixation reaction. The agar gel technique proved to be unreliable as a supplementary diagnostic test.

A free soluble antigen of Anaplasma marginale, exo-antigen, was found to be produced and released into the peripheral blood of cattle with acute anaplasmosis. The significance or immunogenic potential of this material has not been determined.

Calves given an immunizing inoculation of sonicated hemolysate of anaplasma-infected RBC (red blood cell) in a mineral oil adjuvant and then challenged were only partially protected. The hemolysate conferred partial protection against the severe form of the disease but did not prevent the animals from becoming carriers.

Filtration and high speed centrifugation with a sucrose gradient were employed in the examination of the complement-fixation antigen to determine whether sub-microscopic particles of Anaplasma occur in this antigen. Sub-microscopic units of A. marginale were not found.

Ten adult-to-larva and two series of adult-to-adult hereditary transmission experiments with the Rocky Mountain wood tick, Dermacentor andersoni Stiles, failed to transmit anaplasmosis. All test calves were found to be susceptible when challenged with anaplasma-infected blood.

Exposure of adult ticks to hibernating environments of 4°C, relative humidity of 40-50%, and of 25°C, relative humidity of 80%, had no observable effect on their transmission potential. However, ticks exposed to the low temperature fed faster and produced 50 - 70% more eggs after hibernation than did ticks held at 25°C. Nine of 49 D. andersoni males, subjected to hibernation for 263 days, survived. Of these, 5 attached and fed on a susceptible calf which failed to contract anaplasmosis. The ticks had originally fed on a calf with acute anaplasmosis 358 days before they were placed on the test (susceptible) calf.

Colonization of D. occidentalis has been successful and the colony is now in the F_3 generation. (Maryland)

The ARS anaplasmosis research herd at Kerrville, Texas, is composed at the present time of 40 mature cows, 8 two-year-old heifers, and 27 calves nearing weaning. The last of the reactor cattle were sold last year (1963) and the herd has continued through this fiscal year as an anaplasmosis-free herd. The total number of cattle will be reduced to approximately 35 to conform to available pasture. (Texas) (ADP b1-30)

L. Interrelationship of Diet and Parasitic Infection in the Production of Cattle.

Research workers at the ADP Regional Laboratory, Auburn, Alabama, reported the effect of parasitosis on the basal metabolic rate of rabbits infected with either 5,000, 10,000, 15,000, 20,000, or 25,000 infective Obeliscoides cuniculi larvae, indicated that the parasitic infections established did not cause a marked difference between the basal metabolism of the infected and control animals.

Experiments on the biology and host-parasite relationship of Longistrate noviberiae and Trichostrongylus affinis in domestic rabbits indicate these parasites are well adapted for experimental use. The short prepatent period, direct life cycle, high percentage of adult worm recovery makes these ideal parasites for experimental work with diets in rabbits. (Auburn, Alabama) (ADP b1-31)

M. Histochemistry of Gastro-Intestinal Nematodes of Cattle.

The report on work conducted at the ADP Regional Animal Disease Laboratory, Auburn, Alabama, showed that 8 to 10 days after infection of cattle with the nodular worm, Oesophagostomum radiatum, there is a decrease in collagen around the lesions formed by the worm in the walls of the ilium. Concurrently with this decrease in collagen there is a real or apparent increase in a substance, probably glycoprotein, containing protein and carbohydrate moieties, between the lesions and the lumen of the intestine. Such histochemical changes were not observed around the sites of infection by the medium stomach worm, Ostertagia ostertagi, 8 days after the host calves were infected. At the above stages of these diseases, alterations in the distribution of glycogen and acidic mucopolysaccharides of the tissue were not observed.

Glycogen and acid mucopolysaccharides, as well as collagen, have been found in Obeliscoides cuniculi, a nematode of the domestic rabbit. Glycogen was principally found in the intestinal wall and in the body wall muscles. Acid mucopolysaccharides lined the digestive tract and were present in the muscles of the body wall. Collagen was seen in the cuticle, the hypodermis, and in various membranes, as well as in the gonads. (Alabama) (ADP bl-32)

N. Parasites of Cattle with Emphasis on Stephanofilarial Species.

Investigations made at the ADP Regional Animal Laboratory at University Park, New Mexico, were reported as follows: Stephanofilaria stilesi is a small filarioid nematode causing an ulcerative dermatitis along the ventral mid-line of cattle, and uses the horn fly, Haematobia irritans, as a biological vector. Horn flies were experimentally infected with the larval stages of S. stilesi by exposing laboratory-reared flies to the lesion on infected cattle. The infective stage is reached after about 18 days of development in the fly. The biological cycle of S. stilesi was completed by exposing young calves to infected horn flies. Two calves developed lesions typical of stephanofilariasis within two weeks after their initial exposure. One calf was examined post-mortem after one month and found to be infected with immature S. stilesi. From eight to thirty-two per cent of the horn flies collected from infected cattle on rangeland, irrigated pasture, and in drylot were found to be infected with the larvae of S. stilesi. (New Mexico) (ADP bl-33)

O. Under a PL 480 Grant to the School of Veterinary Medicine, University of San Marcos, Lima, Peru, research is in progress on Environmental Factors Influencing Parasites and Parasitic Diseases of Economical Importance in Ruminants (Cattle-Sheep-Alpacas). Most of the work reported has been of the nature of a preliminary survey of multiple areas or districts to determine the kinds of parasites therein that infect animals.

P. Under a PL 480 Grant to the School of Veterinary, Montevideo, Uruguay, research is in progress on Anaplasmosis, Piroplasmosis, and Babesiellosis of Cattle. Crushing and macerating of tick larvae appeared necessary for development and evolution of the larvae, and their transformation into nymphos in vitro. A temperature of 37°C was found to be the most favorable for cell survival and development.

The development of tissular components was improved by the addition of glutamine to the media. Chicken plasma was also found to improve the media.

The pathogenicity of whole blood infected with Babesia bigemina was modified by irradiation with gamma rays at dosages above 30,000 r.

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AREA NO. 12 - PARASITES AND PARASITIC DISEASES OF SWINE

Problem. Parasitic diseases have been estimated to cost the swine industry of the United States at least $200 million annually. These diseases for the most part are cosmopolitan. Subclinical infections are the most frequent type and the most costly, yet they are generally so difficult to recognize that they often are overlooked entirely. Diagnosis is difficult, and successful treatments for many of these parasitisms are not available. Moreover, management practices to avoid the spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling, or eradicating parasitic diseases so as to provide for healthy swine, insure adequate supplies of parasite-free pork for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a prosperous agriculture, a sound national economy, a high standard of living, and a healthy population.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving parasitologists, veterinarians, biochemists, microbiologists, and pathologists engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 4.7 professional man-years. This effort is divided among sub-headings as follows:

The role of parasites in the economy of swine production 1.2 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and at the Division's laboratory at Tifton, Georgia, through informal cooperation with the Georgia Coastal Plain Experiment Station.

Bionomics and pathogenicity of the swine whipworm 0.5 at the Beltsville Parasitological Laboratory.

Swine kidney worms 2.1 at Tifton, Georgia, the Beltsville Parasitological Laboratory, and under cooperative agreement with the North Carolina Agricultural Experiment Station at Raleigh.

Investigations of Trichinella spiralis 0.5 at the Beltsville Parasitological Laboratory.

Effect of anthelmintic treatment on rate of gain 0.3 at Tifton, Georgia.

Pathogenic role of the intestinal roundworm 0.1 under a cooperative agreement with the Nebraska Agricultural Experiment Station at Lincoln.

PROGRAM OF STATE EXPERIMENT STATIONS

Six States have studies concerned with various phases of the internal parasite problem in swine. Part of this work is carried on under cooperative agreements with the Department. The major effort is centered on swine ascarids. Efforts are being made to develop methods of immunization or treatment which will prevent damage caused by migration of ascarid larvae through the body. Germ-free studies seek to establish the exact pathology caused by ascarids in the absence of other organisms. An evaluation is being made of the role which these parasites play in rendering swine susceptible to infectious diseases.

The migration patterns of kidney worms through swine are being traced and the resulting damage determined. Control and eradication procedures through management practices are being developed for this parasite. Anthelmintics are being evaluated for the control of Strongyloides infection in swine.

1.4 professional man years of scientific effort are devoted to swine parasite research at the States.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Swine kidneyworm

At the Animal Parasite Laboratory, Tifton, Georgia, a program for the eradication of kidneyworm, Stephanurus dentatus, from a naturally infested area was started on a private farm near Nashville, Georgia, in the fall of 1960. The management system consisted of breeding only gilts to farrow pigs and removing the gilts once their pigs had been weaned. From an infection of 93% of pigs farrowed by gilts from an infested area in the spring of 1961, the incidence of kidneyworms dropped to 50, 18, 6, and 0%, respectively, in succeeding semi-annual farrowings. All pigs farrowed by gilts raised either in the original kidneyworm-free area, or the original kidneyworm-infested area, were negative for kidneyworms in the spring of 1963. (Tifton, Georgia)

At the North Carolina Agricultural Experiment Station, Raleigh, prenatal infection with kidneyworm was accomplished in each of 4 trials. This represented repeated doses of infective larvae during the length of pregnancy. It is felt that prenatal infection would be the reason why many young pigs in endemic areas show well developed parasites when they are slaughtered at 5-7 months of age. (Raleigh, North Carolina) (ADP b2-11(Rev.)

B. Intestinal threadworm

At the Animal Parasite Laboratory, Tifton, Georgia, infection of baby pigs with Strongyloides ransomi has previously been shown to be the most serious parasitic problem in swine of the South Georgia and North Florida area. Experiments have demonstrated prenatal infection of pigs with S. ransomi. This finding throws new light on the bionomics of the host-parasite relationship and may lead to new ways for control. Exposure of weaned pigs to infective larvae of S. ransomi established that 1 million larvae was usually sufficient to produce a marked reduction in the weight gains of infected pigs. (Tifton, Georgia) (ADP b2-17)

C. Intestinal roundworm

At the Animal Parasite Laboratory, Tifton, Georgia, observations have been made on the effects of anthelmintic treatment of pigs infected with Ascaris suum. Some of the trials were made with naturally infected pigs, others with experimentally infected pigs. Results of these experiments have been erratic and unpredictable. During fiscal year 1964, the research effort was directed toward making observations on the effects of adequate and inadequate rations on pigs infected with Ascaris. The adequate ration contained 16% protein and the inadequate 14 percent. It was found that the effect of migration of 125,000 Ascaris larvae was not as pronounced in reducing the daily gain of pigs as was the reduction in percentage of protein in the inadequate feed. (Tifton, Georgia) (ADP b2-4(Rev.)

At the Nebraska Agricultural Experiment Station, Lincoln, injection of serum, from pigs actively immunized by repeated administration of infective eggs, induced immunity in non-exposed pigs. This demonstrated that the immune factor circulates in the serum and can be extracted and used as an immunizing agent. Several new compounds were checked as ascaricides in a field trial. Piperazine was the only one that effectively killed worms. The others were either non-efficacious, non-stable, or were added at too low a level for action. The intraperitoneal implantation of a resin-coated organic phosphate checked the migratory stage of ascaris but tissue reaction precludes its application. It was found that migrating ascaris enhanced the severity of swine influenza; 90 per cent of mice infected with both agents died, whereas only 30 percent of those with only influenza died. The virus multiplied more rapidly in the doubly infected mice. Migrating ascaris caused severe liver lesions in swine. It was found that the liver completely recovers from this damage within 35 days. (Lincoln, Nebraska) (ADP b2-12(Rev.)

D. Swine whipworm

Continuing studies at the Beltsville Parasitological Laboratory, have shown that the eggs of Trichuris suis, the swine whipworm, have remained infective to susceptible pigs for approximately 8 years when exposed on the surface or when buried 4 or 8 inches in sandy loam soil. (ADP b2-10(Rev.)

E. Trichinellosis

Studies at the Beltsville Parasitological Laboratory showed that all trichinae in pork cuts weighing from 10 to 45 pounds were killed by a 20-day exposure in a 9 cubic-foot, chest type, home freezer, filled to capacity with 319 pounds of meat and set to operate at 0°F. Trichinae in 1-pound patties of ground pork were rapidly killed by exposure to 0°F in a home freezer. Exposure for 18 hours destroyed from 43 to 65 percent of the larvae. After 138 hours living trichinae were not found in the 1-pound patties, but 2 were found in a pail containing 13 pounds of ground trichinous meat.

Some evidence was obtained to indicate that the resistance of trichinae to the effects of freezing may be increased by prior exposure to 35°F for periods of 51 and 135 days.

Infection with trichinae occurred in non-trichinous pigs from the ingestion of feces of donor pigs containing larvae of this parasite passed within 4 days after the ingestion of trichinous meat.
(Beltsville, Maryland) (ADP b2-15)

Investigations on trichinellosis are also being conducted under a PL 480 grant to the Polish Academy of Science, Warsaw, on the epidemiological, epizootiological, and immunological aspects of this disease to establish information on the incidence of Trichinella spiralis in people and domestic and wild animals throughout the country. Allergic tests for diagnosis of the disease are being assessed. Other studies indicate that the intestinal flora in the host's digestive tract may affect the invasive ability of the larvae. (Poland) (E21-ADP-9)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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AREA NO. 13 - PARASITES AND PARASITIC DISEASES OF SHEEP AND GOATS

Problem. The cost of parasitic diseases to the sheep and goat industry of the United States is estimated to be in excess of $45 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult, and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy animals, insure adequate supplies of high quality lamb for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a prosperous agriculture, a sound national economy, a high standard of living, and a healthy population.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, parasitologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of parasites and parasitic diseases of sheep and goats. Research is being conducted on these diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 10.2 professional man-years. This effort is divided among sub-headings as follows:

Bionomics of Coccidial Parasites 2.0 at the Beltsville Parasitological Laboratory.

Effects of Helminth Infections on Serum Proteins 0.5 at the Beltsville Parasitological Laboratory.

Gastrointestinal Nematodes 2.1 at the Beltsville Parasitological Laboratory, and under a cooperative agreement with the Kentucky Agricultural Experiment Station at Lexington.

Helminth and Protozoan Parasitism in the South 1.5 at the Regional Animal Disease Research Laboratory, Auburn, Alabama, and through informal cooperation with the Mississippi Agricultural Experiment Station, State College.

Biology, Pathogenesis, and Control of Helminth Parasites of Sheep in the Southwest 2.0 at the University Park, New Mexico, field station, and through informal cooperation with the New Mexico Agricultural Experiment Station at University Park.

Effect of Intestinal Roundworms on Metabolism 0.1 under cooperative agreement with the North Dakota Agricultural Experiment Station, Fargo.

Control of the Common Sheep Scab Mite 2.0 at the Albuquerque, New Mexico, field Station.

PROGRAM OF STATE EXPERIMENT STATIONS

The majority of applied research in this area at the States involves sheep rather than goat parasites. Work is closely interrelated with parasite research in cattle and much of the basic work is applicable to cattle, sheep and goats.

Regional project W-35, previously mentioned under Area #11, serves to coordinate the work on sheep and goat parasites which is in progress in the Department and in the Western States. This group maintains informal cooperation with southern States working on this problem.

Information is being obtained concerning the effects on parasitism of climate, types of pasture grasses, stages of plant growth, rates of stocking, methods of supplemental feeding and early or late lambing. Several States are determining how the genetic background of sheep affects resistance or susceptibility to parasites. New anthelmintics and larvaecides are being evaluated to provide improved control measures.

Other studies are aimed at finding the source of infection and life cycle of the fringed tapeworm of sheep. Several States are evaluating chemical control and other procedures for reducing parasitic infection from liver flukes.

A number of States have basic work in progress on the physiological changes which occur in the host during parasitic infections. Other work is concerned with measurement by serological methods the immune response occurring in parasitized sheep. Radio-isotope techniques are being used to determine the effect which parasites have upon the absorption of essential food elements.

The States have 4.1 professional man-years of research involved in sheep and goat parasite research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Bionomics of Coccidial Parasites

At the Beltsville Parasitological Laboratory detailed studies on swollen mesenteric lymph nodes of sheep and goats infected with the coccidia Eimeria arloingi and E. ninaekohlyakimovi gave evidence that the schizont or early developmental stage of one or both of the parasites commonly occurs in the mesenteric lymph nodes and that edema fluid rather than the presence of the parasites is probably the primary cause of the enlargement of the nodes. (Beltsville, Maryland) (ADP b3-14)

B. Helminth and Protozoan Parasitism in the South

At the Regional Animal Disease Research Laboratory at Auburn, Alabama, pure cultures of oocysts of Eimeria intricata were obtained by micromanipulator isolation and feeding of small numbers to three lambs. These pure cultures were used in infection studies and in an attempt to determine the life cycle of this species which twice has been suggested as the one that produces the large (up to 700 μ) schizont-like bodies in the abomasa of sheep in various parts of the world.

The prepatent period in heavy, mixed, and pure infections ranged between 20 and 23 days, with a patent period of 6 to 11 days. In fresh smears, no endogenous stages were found at 8 days post-inoculation, but schizonts and merozoites were found 12, 15 and 18 days. The largest schizont measured only 52 x 58.5 μ but the largest merozoite was 3.9 x 20.8, giving a coarsely granular appearance to the mature schizonts. Gametocytes and immature oocysts were found in the lower small intestine. Nothing was found in the stomachs, cecum, or colon. Photomicrographs of the reported endogenous stages were made and sections are now being processed. (Auburn, Alabama) (ADP b3-19)

At the Auburn Regional Research Laboratory, oral inoculation of guinea pigs with 5,000 Trichostrongylus colubriformis infective larvae, followed by therapeutic termination of infection 2 days later, and intraperitoneal injection of 5,000 artificially exsheathed infective larvae, provided guinea pigs with protection against reinoculation. Single inoculations and injections were as effective as two inoculations or injections administered one week apart. (Auburn, Alabama) (ADP b3-21)

C. Biology, Pathogenesis, and Control of Helminth Parasites of Sheep in the Southwest

At the University Park, New Mexico, station, results from a preliminary experiment involving 18 lambs indicated that inoculation with an attenuated antelope strain of Haemonchus, a common stomach worm, followed 34 days later by treatment of phenothiazine, produced as effective a resistance to challenge with sheep strain Haemonchus as did inoculation not followed by treatment. It is postulated that drug treatment to remove immunizing infections after they have acted would be advantageous under practical conditions in order to prevent undue contamination of pastures.

Studies on the life history of Elaeophora schneideri, the arterial worm of sheep and deer, indicate that sheep are abnormal hosts and deer are normal hosts. Seven sheep, each having bloody lesions as a result of infection with this nematode, were examined for microfilariae of E. schneideri. Microfilariae could be demonstrated only intermittently in skin from the lesions of two animals. At postmortem examination of the five not showing microfilariae, only non-gravid females were found. Two mule deer and one white-tailed deer collected near Roosevelt, Arizona, were infected with E. schneideri. All had numerous microfilariae in the skin about the poll and face; none had any of the lesions so characteristic of infected sheep.

In investigations of the mode of transmission of the fringed tapeworm, a common parasite of western sheep, efforts were concentrated on insects known as bark lice, or psocids. These insects were collected on sheep range and fed to tapeworm-free lambs in an attempt to produce infections; the results were negative. Six species of psocids were cultured in the laboratory and exposed to tapeworm eggs; 169 tapeworm larvae were recovered from a total of 46 exposed psocids, but these failed to infect the four test lambs to which they were fed.

Of 53 sheep examined for liver flukes from southern Colorado and northern New Mexico, 15 were positive. Six species of snails from these areas were identified. Two of these species, Fossaria modicella and Stagnicola bulimoides techella have been incriminated as intermediate snail hosts in other areas.

The compound Bayer ME3625 was found to be effective in removing adult liver flukes from sheep when used at the rate of 300 or 450 mg/sheep but Neguvon at the rate of 100 mg/kg was ineffective. Absence of immature flukes prevented evaluation of the two drugs against these forms. Geigy compound GS 27384, was found to have some effect against the fringed tapeworm when used at a rate of 300 mg/kg, but this chemical was found to be quite toxic. Bayer 2353 removed 100 per cent of the tapeworms from 11 sheep treated at the rate of 600 mg/kg, while 10 untreated controls harbored an average of 27.9 tapeworms each. There were some mild signs of toxicity in treated animals, the importance of which remains to be evaluated.

New information is provided concerning parasites of mule and white-tailed deer in Arizona and New Mexico, bighorn sheep in Nevada, and javelina and jack rabbits in New Mexico. Trichostrongylus colubriformis, an extremely prevalent intestinal worm of sheep, was found in both javelina and jack rabbits, indicating that these animals may serve as reservoir hosts of this parasite. (University Park, New Mexico)(ADP b3-18)

D. Effect of Intestinal Roundworms on Metabolism

In cooperation with the North Dakota State Agricultural Experiment Station, Fargo, the effect of gastrointestinal nematodes on the tensile strength and sulfur content of wool was studied and the response of these factors to sulfur supplementation in the feed was also investigated. Four groups of eight lambs each were utilized. They were: 1) non-infected, non-supplemented; 2) non-infected, supplemented; 3) infected, non-supplemented; 4) infected, supplemented. All infected lambs were given 50,000 infective larvae of gastrointestinal nematodes, primarily Trichostrongylus sp. perorally by capsule. Supplemented lambs received sodium sulfate in the feed to provide 1 pound of sulfur per ton of feed. Infected lambs gained less weight during the trial period, but there was no apparent difference in tensile strength or sulfur content of the wool between the groups. The gastrointestinal nematodes were collected at the termination of the trial period and most of the infected lambs had heavy worm burdens.

There appeared to be no correlation between either worm load and sulfur content of wool, or worm load and tensile strength of wool. This was contrary to results of some earlier studies. There was an apparent depression of nematode infection with sulfur supplementation.
(North Dakota) (ADP b3-20)

E. Control of the Common Sheep Scab Mite

At the Albuquerque, New Mexico, field station, nine tests, involving groups of sheep heavily infested with Psoroptes ovis, numbering from 26 to 56 animals, were conducted on 4 candidate acaricides. All four products, in any concentration used, failed to eradicate infestations of the parasite. It was speculated that the reason for the failure of Co-Ral, cold lime-sulfur, Korlan and Ciodrin, three of which had previously proved successful in controlling sheep scab, was the temper and constitution of the test animal. The test subjects in this case consisted of highly pathogenic and resistant field strains of mites, including one designated "Corona", which was more vigorous, pathogenic and aggressive than any yet encountered at this laboratory.

Candidate and established acaricides were applied as a dip to 9 groups of uninfested sheep which were then challenged by being placed in a pasture with 40 sheep heavily infested with Psoroptes ovis. A group of 20 undipped, uninfested controls was also challenged in the same manner. The untreated sheep all became infested in from 13 to 34 days after challenge. The

dipped sheep were protected for periods ranging from 28 to 154 days. Most effective in its residual effectiveness was Toxaphene, followed by Ciodrin. Least effective was Delnav, Co-Ral, and a Korlan formulation.

Both single and double applications of unheated lime-sulphur dips, containing 1.75% calcium polysulfides, eliminated scabies infestations from heavily infested sheep. Success of treatment, however, was tempered by the fact that an avirulent strain of mites was involved in these trials. It was noted, following a subsequent test, that a single application of cold lime-sulphur dip at the above concentration of toxicant failed to eradicate an infestation of a highly pathogenic strain of P. ovis.

Observations were made at Albuquerque on the comparative pathogenicity of various strains of Psoroptes ovis. Recent experiences with a variety of strains have contributed much information to what has been suspected for several years regarding this subject. It has been shown that a) acaricides effective against avirulent strains of P. ovis may fail to control pathogenic strains; b) sheep harboring avirulent strains do not acquire resistance to virulent strains, c) pathogenic strains of mites appear to be highly refractory to chemical control, and d) avirulent strains are known to recede into a period of summer latency, during which time their existence is extremely difficult to establish, while pathogenic strains can be responsible for active acariasis well into or throughout the summer season. These observations have altered our concepts regarding the evaluation of acaricides for the control of scabies, and should contribute to the success of efforts to control scabies on a nationwide basis.

During investigations into the latency of Psoroptes ovis during the summer months, the questions as to where, and under what circumstances P. ovis oversummers, particularly in areas where summer atmospheric temperatures are high, appears to be nearing resolution. Continued studies into the oversummering locations revealed that a) mites apparently oversummer, in large measure, on the broad body surfaces of sheep, in locations where they displayed activity and produced skin injury during the previous spring, before entering into summer dormancy. Inspectors in search of evidence of scabies during the summer months are therefore advised to examine old, healing lesions as probable areas of involvement. b) there is no reason to suspect, at least in the Southwest, that mites are likely to escape direct contact with acaricides if dipped during the summer season, by virtue of their inaccessibility in "summer hiding places," such as the various cutaneous orifices. (Albuquerque, New Mexico) (ADP b3-22)

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General

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(PL 480 Grant)

AREA NO. 14 - PARASITES AND PARASITIC DISEASES OF POULTRY

Problem. Parasites and parasitic diseases probably cost the poultry industry many millions of dollars annually by causing intestinal disturbances, emaciation, retarded growth, reduced egg production, and deaths. Parasites are ubiquitous, many times insidious, and often overlooked until birds are damaged irreparably. Early diagnosis is difficult, and reliable treatments for many devastating parasitoses are not available. Moreover, some management practices, intended to avoid spread of parasites and to control them, have been found ineffectual as is shown by the increasing importance of certain parasites in broiler production. The problem is to develop, through a planned, balanced program of basic and applied research, methods for preventing, controlling or eradicating parasitic diseases, thus affording economical production of healthy poultry and sound products in supplies adequate to meet the needs of an expanding population.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving parasitologists, biologists, and chemists, engaged in both basic studies and the application of known principles to the solution of the problem of parasites and parasitic diseases of poultry.

The Federal scientific effort devoted to research in this area totals 5.5 professional man-years. This effort is applied as follows:

Control of Coccidiosis 2.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Biology of Nematode Parasites 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Biological investigations of protozoan parasites and parasitic diseases, with special reference to those of the gastrointestinal tract 2.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

PROGRAM OF STATE EXPERIMENT STATIONS

The principal effort being placed in this area by the States is on coccidiosis. Factors influencing immunity to this disease are being determined and prevention of outbreaks by means of vaccination is being evaluated. The role of nutrient elements in affecting severity of the condition is under study. Several States have work in progress on the blackhead parasite of poultry to learn methods of building resistance against this organism. Other research is in progress to determine life cycles of poultry tapeworms and capallarids and to determine the effects of these parasites on egg production, weight gains and feed efficiency.

There are a total of 2.0 professional man-years allocated to research on parasites of poultry at the States.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Biology of Nematode Parasites

Research at the Beltsville Parasitological Laboratory revealed that a mash containing 0.5 percent thiabendazole removed all gapeworms, Syngamus trachea, from 97 percent of 67 turkey poults that were given the medicated feed for 9 to 20 days. In the aggregate, the regimen removed 96 percent of the total worm populations in the treated birds and was well tolerated in all respects. Systemic action of the drug was indicated by its anthelmintic effect against worms that had migrated from the intestine to the trachea before treatment was started. (Beltsville,Maryland) (ADP b4-10)

B. Biological Investigations of Protozoan Parasites

At the Beltsville Parasitological Laboratory the following work was conducted: As evidenced by oocyst output and extent of lesions following experimental inoculation of similar doses of oocysts, 1-day-old chicks were less susceptible to infection with the coccidium, Eimeria acervulina, than were 3-day-old chicks. This is considered to be due, at least in part, to the inability of the gizzards of 1-day-old chicks to break the walls of the oocysts which are tough and more-or-less impervious. In chickens 3 - 44 days of age, the oocysts were broken in the gizzard and the liberated sporocysts passed on into the intestine where the sporozoites were activated to escape from the sporocysts. The gizzards of 1-day-old chicks have poorly developed musculature and smooth inner linings, whereas the gizzards of 3-day-old chicks are relatively muscular and are lined with a rough material (koilin). Examinations of gizzard and intestinal contents of chicks previously inoculated with massive doses of oocysts showed that much higher percentages of sporocysts were liberated in the gizzards and much higher percentages of sporozoites excysted in the small intestines of 3-day-old chicks than in 1-day-old chicks.

Three species of earthworms have been found to be true, but not obligatory, vectors in the transmission of Heterakis gallinarum, the cecal worm of chickens, turkeys, and ring-necked pheasants, and in the transmission of the protozoan, Histomonas meleagridis, the causal agent of blackhead.

The pancreatic enzymes necessary for excystation of Eimeria acervulina, and perhaps all other poultry coccidia, are trypsin and chymotrypsin. In the presence of a bile salt, these enzymes digest the protein-like sporocystic plug and allow the sporozoite to excyst. Lipase and carboxypeptidase, the other pancreatic enzymes, are without effect. (Beltsville, Maryland) ADP b4-11)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Protozoology

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Lund, Everett, and Farr, Marion M. 1964. Protozoa. Chapter in DISEASES of POULTRY, by Biester and Schwarte. Iowa State University Press, Ames.

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Helminthology

Wehr, Everett E., and Hwang, J. C. 1964. The life cycle and morphology of Ascaridia columbae (Gemelin, 1790) Travassos, 1913 (Nematoda:Ascarididae) in the domestic pigeon (Columba livia domestica). J. Parasitol. 50:131-7.

AREA NO. 15 - TREATMENT FOR REMOVAL OF PARASITES OF DOMESTIC ANIMALS

Problem. Parasites of food animals are responsible for losses to livestock producers approximating a billion dollars annually. This estimate, moreover, is conservative since it does not take into account costs of treatment and other control measures. Chemical antiparasitic agents are the most powerful weapons presently available against parasites and the diseases they cause, yet specific treatments generally have a comparatively short period of usefulness. Many of the currently preferred treatments were unknown a decade or so ago and, in all probability few, if any of those in use today will be primary choices a decade or so hence. Moreover, the growing concern with respect to residues in edible tissues and organs of treated animals and birds necessitates development of control measures other than treatment. The problem is to develop, through a planned, balanced program of basic and applied research control methods that minimize reliance on extrinsic chemicals. These include investigations of immunological procedures, management practices which minimize exposure of animals to parasitic infections, and natural control agents such as parasites, pathogenic microorganisms, and predators of economically important livestock pests.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving veterinarians, parasitologists, pharmacologists, and biochemists engaged in both basic studies and the application of known principles in developing treatments for removal or control of parasites of domestic animals. Research is being conducted on this problem at the following designated locations.

The Federal scientific effort devoted to research in this area totals 13.0 professional man-years. This effort is applied as follows:

Chemical Control of Parasitic Diseases 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

New and Improved Anthelmintics 3.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Hazards of Residues from Treatment for Parasites 3.5 at the Regional Animal Disease Research Laboratory, Auburn, Alabama.

Parasitic and Related Skin Diseases 1.5 at the Albuquerque, New Mexico, field station.

Pathobiology of Parasitic Infections 1.0 at the Albuquerque, New Mexico, field station.

Methods for Control and Eradication of Ticks 1.0 at the Albuquerque, New Mexico, field station.

Control and Eradication of Scabies 1.5 at the Albuquerque, New Mexico, field station.

PROGRAM OF STATE EXPERIMENT STATIONS

Research in this area at the States is aimed at providing a scientific basis for chemical control of livestock parasites. A number of States cooperate with the USDA in this research through regional project W-35. New compounds are evaluated singly and in various combinations to determine the most effective treatments against important parasite species. Methods of administering these compounds and dosages required for most effective parasite control are evaluated along with considerations of toxic effects which the compounds may have on host animals. Procedures are being developed to simplify administration of anthelmintics and their use coordinated with effective management practices.

Basic studies at several States seek fundamental information on how anthelmintics act upon metabolic processes of parasites to impair their reproductive processes or result in death. The problem of drug resistance in certain strains of parasites also is being studied.

7.9 professional man-years are being spent on chemotherapy of livestock parasites at the States.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Control of Parasites

The following work was conducted at the Beltsville Parasitological Laboratory: Chemicals with suspected potential anticoccidial activity (dimetridazole, Germall, 5-nitrobenzimidazole, and 5-nitro-2-benzimidazolethiol) were fed to groups of chickens with experimental Eimeria tenella infections. There were no detectable differences between the performance of the experimental birds and infected, unmedicated controls.

Strains of E. tenella with laboratory-induced tolerance for zoalene, glycarbylamide, nitrofurazone, nicarbazin, and Trithiadol, respectively, showed no cross-resistance to amprolium. Strains of E. tenella, passed through 45 successive groups of chicks medicated with Unistat or arsenosobenzene, respectively, developed a pronounced tolerance to the specific drugs. The degree of tolerance was greater in the Unistat-medicated strain than in that exposed to arsenosobenzene.

A laboratory strain of Eimeria tenella that was serially exposed to nicarbazin for 40 generations developed a degree of tolerance to the drug sufficient to result in mortality among nicarbazin-medicated birds. It had, however, no significant cross-resistance to Trithiadol, Unistat, zoalene, nitrofurazone, arsenosobenzene, or glycarbylamide.

A commercial litter spray, formulated from cresylic acid and a petroleum distillate, had no adverse effect on Eimeria tenella oocysts. Neither sporulation rate nor virulence was reduced as a result of the application of this product.

Elimination of all Trichomonas gallinae organisms from pigeons that had been harboring them with no apparent ill effects resulted in loss of immunity to trichomoniasis caused by T. gallinae. Severe mortality resulted when pigeons that had been trichomonad-free for six days were infected with a new strain of T. gallinae.

Trichomonas gallinae from the upper digestive tract of pigeons was successfully established intravaginally in hamsters. T. gallinae recovered from these hamsters infected 60 percent of the pigeons exposed to it. T. foetus from hamsters or bulls, however, failed to become established in pigeons.

Only one of nine chickens, and none of four turkeys, maintained for over a month in intimate contact with pigeons harboring Trichomonas gallinae, acquired an infection of this parasite. In contrast, T. gallinae rapidly spread from infected to uninfected pigeons housed under similar conditions.

Histomonas meleagridis that had been cultured in vitro for 5 years, grew in young chickens and turkeys following rectal inoculation. The infections, however, were innocuous and conferred no immunity against a challenge inoculation with a highly virulent strain of the parasite.

Studies were continued on the effect of various management systems, including medication, on the development of parasitism in lambs. Four bands of approximately 40 lambs each were raised under varying degrees of exposure to gastrointestinal parasites. Band 1 lambs were weaned at 60 days of age and grazed on newly renovated, uncontaminated pastures. Band 2 lambs were grazed on relatively "clean" pastures. Band 3 and Band 4 lambs were grazed on separate, contaminated pastures used by comparable lambs of these bands the previous year. All bands were moved at bi-weekly intervals, or as required by available forage, among pastures assigned to each band. Lambs of Bands 1, 2, and 3 had continuous access to a 1:9 phenothiazine-mineral mixture, and all animals within a band were given therapeutic doses of purified, micronized phenothiazine when parasite egg counts approached 1,000 eggs per gram of feces. The lambs of Band 4 were given continuous access to unmedicated mineral mixture; therapeutic doses of thiabendazole were given on the same basis as that followed in treating lambs of Bands 1, 2, and 3 with phenothiazine.

Analyses of data, including hematocrit determinations, weight gains, parasite egg counts, and necropsy worm counts, showed 1) that lambs on newly renovated pastures remained essentially parasite free and made excellent weight gains; 2) lambs on "clean" pastures acquired light infections but required no therapeutic treatments; and 3) lambs on contaminated pastures developed stomach worm infections despite free-choice and therapeutic medication with phenothiazine (Band 3) and therapeutic dosing with thiabendazole (Band 4). The data indicated that thiabendazole was less effective than phenothiazine against the predominant pathogen, Haemonchus contortus, but more effective than phenothiazine against Strongyloides papillosus. Data on other worm parasites were too limited to permit fair comparisons of efficacy. (Beltsville, Maryland)

In studies at the Regional Laboratory, Auburn, Alabama, an anthelmintic test in lambs at Natchez, Mississippi, failed to show any significant differences in worm numbers between groups treated with phenothiazine, Thibenzole, and Promintic with the exception of numbers of Haemonchus contortus which were significantly lower in the group treated with phenothiazine. All 3 treatment groups differed significantly from the control group in numbers of Trichostrongylus axei, T. colubriformis, and Hematodirus spp. found at necropsy.

After a lapse of 30 days between date of treatment and necropsy, the group treated with phenothiazine had significantly fewer numbers of Cooperia spp.; no other real differences occurred among the treatment groups.

A test of Bayer 9002, N-Hydroxynaphthalimide diethyl phosphate, at dose rates of 25 and 50 mg/kg demonstrated significant differences in numbers of Haemonchus contortus and Ostertagia circumcincta when compared with untreated controls. No other significant differences were found between the treated groups and the controls. No differences were found between the 2 levels of Bayer 9002. (Auburn, Alabama) (ADP b5-5(Rev.)

B. Investigations of treatments for bovine venereal trichomoniasis

At the Beltsville Parasitological Laboratory, research showed that the systemic use of dimetridazole against Trichomonas foetus (the causative agent of a major reproductive disease of cattle) continues to show unusual promise. Two bulls with experimental T. foetus infections were treated effectively with dimetridazole administered orally, and trichomonads have not been found on post-treatment examinations of three other infected bulls that were given the chemical intravenously at a much lower dosage level. The infection in one additional bull failed to respond to an initial low level oral regimen, or to subsequent treatment at higher levels.
(Beltsville, Maryland) (ADP b5-9(Rev)

C. Investigations to develop new and improved chemical agents for the treatment, prevention, or control of helminthic parasites in farm animals

In studies at the Beltsville Parasitological Laboratory, critical data were obtained on the anthelmintic action of piperazine magnesium sulfate, piperazine citrate, and piperazine dihydrochloride against Ascaris lumbricoides and Oesophagostomum dentatum in swine. The chemicals were given in the regular ground feed ration at concentrations of 0.2 per cent for 2 days and 0.6 per cent for 1 day. Both regimens resulted in very effective anthelmintic action against the large roundworm, Ascaris lumbricoides, and the nodular worm, Oesophagostomum dentatum. The drug consumption data showed, however, that when piperazine is given at 0.6 per cent of the feed for 1 day, pigs consumed up to twice the amount of piperazine usually required to achieve effective parasite control, namely, 50 milligrams per pound of body weight during a 24-hour period. (Beltsville, Maryland) (ADP b5-18)

D. Control of Internal Parasites of Livestock by Management Practices that will not create Consumer Residue Hazards

Studies at the Regional Laboratory at Auburn, Alabama, showed that when groups of lambs and hogs were grazed separately and together, in Mississippi, no differences were noted in parasite population in the lambs. Both groups of lambs developed clinical symptoms of parasitism simultaneously. Eight of the 10 hogs grazing with the lambs were found to harbor Trichostrongylus colubriformis in number ranging from 40-440, averaging 122 worms per hog. No worms were found in the hogs grazing alone. No differences in weight gains were noted between the hogs grazing with the lambs, and those grazing by themselves.

Two tests of 104 and 88 days were conducted using 5.5% ronnel in either a granular mineral mixture or in a mineral block form for the control of horn flies. In one test ronnel was compared with a 7.5% phenothiazine-salt mixture and in the other test Co-Ral spray was used for comparison. Phenothiazine gave no significant reduction in horn fly numbers, while a low-level consumption of ronnel resulted in 89 and 96% control. Cattle grub numbers were low but reduction was apparent in the ronnel-treated groups. Ronnel appeared to have some anthelmintic effect as determined by nematode egg counts. Phenothiazine failed to reduce the number of nematode eggs. (Auburn, Alabama) (ADP b5-16)

E. Control and Eradication of Scabies

Investigations at the Albuquerque, New Mexico, laboratory, on sheep and cattle scabies, revealed that the parasite is passed directly from an infested ewe to her lambs. Trials to transfer the mites from sheep to rabbits and rats were not successful. It appears that the mites do not cause much damage to the skin itself - mild itching - but does cause ragged, matted fleece, damaged fibers, and actual loss of wool.

Studies have revealed the presence of cattle scabies on ranches in New Mexico, and in feedlot herds in Texas. Indications are that scabies is caused by a widespread parasite, although natural transmission is slowly accomplished. An infested cow failed to infest 4 clean cows and calves over a 1-year period.

It has been observed at the Albuquerque laboratory that when scabies-infested sheep are placed in isolation, infestations become extinct (so far without exception) in from 4 to 18 months. Inquiries into this phenomenon, attributed to diminished vitality in homogeneous strains of mites, are being continued. A total of 17 sheep are now in isolation pens, some out of doors, others in a solar-lighted, air-cooled building, and others in a darkened, air-cooled building. The influence of summer dormancy on survival, as well as a correlation between strain pathogenicity and survival on isolated hosts, will be studied. (Albuquerque, New Mexico) (ADP b5-15)

F. Pathobiology of Parasitic Infections

At the Albuquerque, New Mexico, laboratory, a test conducted during the past year revealed that ronnel, when administered at a dosage of 25 mg/kg for a period of 36 days to a carrier cow harboring a heavy lice population, effected a less than 50% reduction of lice. Ronnel at this dosage did not depress the red blood count (RBC) level of the blood, diminish the appetite or alter the course of pregnancy in a control cow, or depress the appetite of the principal. Pen and field trials with Ciodrin administered to sheep as a dip, 0.1% toxicant, indicate a high order of effectiveness against keds and leg mange mites. Heavy infestations of these parasites were eradicated by a single dipping. Ear tick control was of a high order.

Ciodrin was found to destroy quickly all motile stages of the foot louse of sheep, but data on its ability to eradicate infestations of this parasite are inconclusive. Its effectiveness against the swine louse appears to be of a high order. In general, Ciodrin appears to show promise as a broad-spectrum ectoparasiticide, and further work with this drug is indicated.

In the course of two surveys of wildlife conducted in New Mexico during the year, live ear ticks were found in 13 of 42 pronghorn antelope. Of 12 javelina, 3 harbored ear ticks, 4 were host to lice, and 3 were infested with an ear mite not yet positively identified. (Albuquerque, New Mexico) (ADP b5-13)

G. Methods for Control and Eradication of Ticks

At the Albuquerque, New Mexico, laboratory, 3 of 26 nymphal ticks (O. megnini) collected from the ears of cattle, have so far survived unfed for 820 days. (ADP b5-14)

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AREA 16 - MISCELLANEOUS PARASITES AND PARASITIC DISEASES

Problem. - Parasitism is a way of life that characterizes the majority of living things. Except for basic life processes, it is probably the commonest biological phenomenon. More than 50,000 kinds of animal parasites (i.e., parasites classified as animals as opposed to those classified as plants) are known. New varieties are being discovered and described at a rate of about 500 per year. Some devastating parasites, indigenous to foreign countries, threaten to surmount barriers imposed against them. Certain of these have already gained new footholds in livestock, poultry, and wildlife. Essential elements of procedure against parasites--established, exotic, or new--are accurate diagnosis, development of full knowledge about them, and research on effective control measures. The primary requirement is development through research of up-to-date knowledge of classification and identification supported by a complete reference collection of parasites, including type specimens and familiarity with global research already done. Basic investigations of parasitisms as biological phenomena are involved, especially in host-parasite relations, immunology, serology, ultrastructure, and other aspects of diagnosis and control. The problem is to develop and maintain up-to-date methods of identification and the essential, supporting reference collections, as well as complete parasitological information extracted from the world's scientific literature; investigate important phenomena and host-parasite systems not covered in specific host categories; and provide bases for detection and control that are adequate to meet existing and anticipated needs, through research on problems involving various parasites and hosts, including wild animals and birds important to agriculture.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program for parasitologists, biochemists, and microbiologists, engaged in basic and applied research in this area. Research is being conducted on the following problems at the designated locations.

The Federal scientific effort devoted to research in this area totals 10.5 professional man-years. This effort is divided among subheadings as follows:

Classification of parasites 2.0 at the Beltsville Parasitological Laboratory.

Maintenance of parasite collection 1.0 at the Beltsville Parasitological Laboratory.

Maintenance and publication of author, subject, and host index-catalogues 2.5 at the Beltsville Parasitological Laboratory.

Immunologic and other biologic approaches to the prevention and control of parasitic diseases 5.0 at the Beltsville Parasitological Laboratory.

PROGRAM OF STATE EXPERIMENT STATIONS

Information is being obtained at a number of locations on the incidence and importance of specific parasites of livestock. Several States have work dealing with morphology and comparative anatomy of parasites to aid in proper identification and classification of these organisms. Basic work on parasites and parasite products is in progress to develop biological methods for control.

7.9 professional man-years are devoted to this area at the States.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Maintenance and publication of author, subject, and host index-catalogues

At the Beltsville Parasitological Laboratory, the Index-Catalogue of Medical and Veterinary Zoology has been maintained and expanded in its various sections: Author, Subject, and Host Catalogues, and Checklist of Specific and Subspecific Names. New entries augmenting the Catalogues are as follows: Author entries, 9,170; Subject entries, 22,322 (including 20,710 Parasite entries, 1,612 Treatment entries); and Host entries, 6,327. The Index-Catalogue has continued to supply references for the Treatment Catalogue of the Antiparasitic Investigations Research Group and to index literature on plant parasitic nematodes. New genera and species of parasites are as follows: Protozoa: 9 n.g., 147 n. sp.; Trematoda: 59 n.g., 345 n.sp.; Cestoda: 26 n.g., 60 n. sp.; Nematoda: 54 n.g., 343 n. sp.; Arthropoda and miscellaneous groups: 43 n.g., 359 n. sp. There have been 167 new citations of periodicals added to the Catalogue. An average of 500 periodicals are examined each day at the National Agricultural Library for parasitological papers to be indexed in the Index-Catalogue.

The Index Catalogue has had more than 80 visitors from the United States and 14 other countries, some of them staying several days, consulting it as a source of information. (Beltsville, Maryland) (ADP b6-5(Rev.)

At the Beltsville Parasitological Laboratory, Supplement 13, Authors: A-Z, of the Index Catalogue of Medical and Veterinary Zoology, was issued in October 1963. Galley and page proof for Supplement 14, Authors: A-Z, was read. Subjects: Trematoda and Trematode Diseases, Part 1: Supergenera and Genera A and B, and Part 2: Supergenera and Genera C were issued in August 1963, and April 1964, respectively. (Beltsville, Maryland) (ADP b6-9(Rev.)

B. Immunologic and other Biologic Approaches to the Prevention and Control of Parasitic Diseases

At the Beltsville Parasitological Laboratory, antigen production of adult male and female Stephanurus dentatus (swine kidneyworm) in vitro was followed by analyzing media collected during prolonged survival. Antigen production was qualitatively identical in both sexes; however, after about three weeks in culture, females produced less antigen than males. Some antigens previously identified in extracts made from freshly isolated worms were absent, indicating that the medium employed may have been deficient in essential nutrients supplied by the host.

Studies were continued on the maintenance of adult Stephanurus dentatus in vitro. Three tissue culture media (NCTC 107, 109, and 199) were used undiluted or in combination with Pitts' medium; Pitts' medium alone served as the reference standard. Only combinations with NCTC 109 allowed survival comparable to that achieved with undiluted Pitts' medium. Worms surviving in media containing NCTC 109 were observed to concentrate a pigmented material, tentatively identified as Vitamin B_{-12}, in specialized cells of the body cavity. Antigen production, as analyzed by gel diffusion tests, was not significantly different in any of the media studied.

Studies were continued on the efficacy of various preparations with demonstrated serological activity as immunizing agents against Stephanurus dentatus. Freshly isolated worms were divided into 2 lots. One lot was used to prepare extracts of the excretory glands and intestines as previously described. The second lot was incubated in screw-capped tubes in Pitts' medium or in undiluted swine serum from a helminth-free animal. After 3 days the worms were removed and the media saved. Four groups of 3 weaned pigs were used in trials with antigens prepared from: 1) excretory gland; 2) intestine; 3) Pitts' medium, and 4) swine serum. In each group, one pig received an intramuscular injection of the appropriate antigen alone, one received the same antigen with complete Freunds adjuvant, and the third served as a control. After 16 days, all were given an oral dose of 1,200 infective larvae and necropsied 30 days later. During the entire experimental period, weekly blood samples were obtained from all animals and the sera reserved for serological study. At necropsy, the livers were removed and the severity of infection evaluated by the number and characteristics of the lesions.

None of the experimental vaccines demonstrated immunizing capabilities. Analysis of sera by gel diffusion showed that animals receiving antigen incorporated in adjuvant had higher titers of antibody to the homologous antigen, but these were without any apparent protective action.

Studies were continued on the in vitro growth of Stephanurus dentatus with the objectives of extending development beyond the fourth stage and of evaluating the influence of various media ingredients. Results were evaluated by comparison with data previously obtained using medium PB-1 enriched with NCTC-109. Experiments with serum substitutes and with various mammalian sera showed that although serum was essential for development to fourth stage, its source and processing (filtration and heating) was not significant. Modifications formulated with various peptone preparations or protein hydrolysates were inferior to the originals. Chick embryo extract produced growth comparable to that obtained with rabbit embryo extract, but media with the former were cloudy and formed heavy precipitates which hindered observation.

None of the modifications without NCTC-109 were comparable to the standard. Addition of NCTC-109 was beneficial in some cases, but considering development obtained, ease of preparation, clarity of medium, and cost of ingredients, none of the modifications surpassed the standard.

Studies were conducted on the serological reactions of ensheathed and exsheathed infective larvae and of advanced developmental stages (third, third molt, fourth, and fourth molt) of the cattle nodular worm, Oesophagostomum radiatum, grown in vitro and exposed to whole sera from bovines singly infected or resistant to O. radiatum or helminth-free. Sera were tested unheated or after heating (56 C for 30 min) and unfiltered or after filtration. Based on the occurrence of two previously described specific reactions, namely, body opening precipitates (BOP) and cuticular precipitates (CP), and on a nonspecific reaction involving coating of the larvae with debris, or clumping of larvae in the absence of debris, it is believed that 4 antibodies account for the reactions observed: 1) a heat stable, filterable antibody that causes BOP on parasitic third stage and third molt larvae; 2) a heat labile, filterable antibody that causes BOP on ensheathed infective larvae and fourth-stage larvae; 3) a heat labile, filterable antibody that causes CP on all stages except exsheathed infective larvae, and 4) a heat labile, non-filterable (from a helminth-free animal), or filterable (from a parasitized animal) antibody that causes debris coating and clumping on all stages. (Beltsville, Maryland) (ADP b6-10)

C. Chemical and physical elements of parasites and parasite-host relationships

At the Beltsville Parasitological Laboratory, in work preliminary to the isolation and purification of individual antigens of swine kidney worms, various organs were dissected from the worms for study. One organ, known as the excretory gland, was shown to contain at least 8 antigens. The intestine contained at least 6 antigens, none of which was common to any one of the excretory gland antigens. The esophagus and body fluid each contained one antigen. In the other organs no antigens were detected.

The excretory gland antigens were further studied by ultracentrifugation. They were found to vary in size and some of the smaller ones passed through ultrafilters. These latter precipitated antibodies from sera of both infected and noninfected swine. Larger antigens did not pass the ultrafilters and were only able to precipitate antibodies from sera of infected swine. The antibodies found in both types of serum were about the same size as the usual normal antibodies in most human and animal serum samples. (ADP b6-11)

D. Maintenance of parasite collections, identification of parasites, and taxinomic investigations.

At the Beltsville Parasitological Laboratory, eight hundred and forty-three (843) lots of specimens (trematodes 162, cestodes 131, nematodes 454, acanthocephalans 10, arthropods 82, and miscellaneous specimens 4) were added to the parasite collection. These include types of many new species and 339 lots of specimens that were collected in Panama. (ADP b6-6(Rev.)

One hundred and forty-seven (147) lots of parasites were identified. Among these were numerous arthropods of medical and veterinary importance that were collected from various animals offered for entry into the United States, including chorioptic, psoroptic, and sarcoptic mites, and several species of ticks (Amblyomma cajennense, A. gemma, A. hebraeum, A. variegatum, Boophilus annulatus, B. microplus, Rhipicephalus evertsi, and R. pulchellus). Several lots of nematodes from mountain goats in Alberta, Canada, were identified and they contained several species that also infect domestic ruminants, including Teladorsagia davtiani, a parasite only recently reported in North America in sheep and reindeer. (ADP b6-2(Rev.)

A checklist of parasites of domestic animals in the United States and possessions, and Canada, was prepared for publication. It lists the common and scientific names of the parasites, location in/on their host, intermediate host, if any, and geographical distribution. One hundred and twenty-one (121) parasites were named from cattle (organisms of uncertain classification 2, protozoans 23, helminths 49, arthropods 47), 119 from sheep and goats (organisms of uncertain classification 2, protozoans 18, helminths 59, arthropods 40), 92 from equines (protozoans 6, helminths 54, arthropods 32), 72 from swine (organisms of uncertain classification 2, protozoans 22, helminths 27, arthropods 21), 132 from cats and dogs (protozoans 15, helminths 66, arthropods 51), and 179 from chickens, turkeys, pigeons, pheasants, ducks, and geese (protozoans 43, helminths 62, arthropods 72).
(Beltsville, Maryland) (ADP b6-12)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Maintenance and publication of author, subject, and host index-catalogues

Doss, Mildred A., Roach, Kathryn F., and Breen, Virginia L. 1963. Index-Catalogue of Medical and Veterinary Zoology, Subjects: Trematode and Trematode Diseases, Part 1: Supergenera and Genera A and B. U. S. Government Printing Office

Doss, Mildred A., Roach, Kathryn F., and Breen, Virginia L. 1964. Index-Catalogue of Medical and Veterinary Zoology, Subjects: Trematoda and Trematode Diseases, Part 2: Supergenera and Genera C. U. S. Government Printing Office

Humphrey, Judith M., and Segal, Dorothy B. 1963. Index-Catalogue of Medical and Veterinary Zoology, Supplement 13, Authors: A-Z. U. S. Government Printing Office

Immunologic approaches to parasitic diseases

Douvres, F. W. 1963. Use of live Oesophagostomum radiatum larvae to detect antibodies in sera and extracts of intestinal tissues from infected or resistant cattle. J. Parasitol. 49(Suppl.): 32

Tromba, F. G., and Baisden, L. A. 1963. Precipitins in sera of swine infected with Stephanurus dentatus. J. Parasitol. 49:633:638

Tromba, F. G., and Baisden, L. A. 1964. Precipitinogens in the excretory gland contents and in extracts of isolated tissues of Stephanurus dentatus. Proc. Helm. Soc. Wash. 31:10-18

Parasite taxonomy

Becklund, W. W. 1963. Lamanema chavezi gen. n. sp. n. and Nematodirus lamae sp. n. (Nematoda: Trichostrongylidae) from the Alpaca, Lama pacos, and the Vicuna, Vicugna vicugna, in Peru. J. Parasitol. 49:1023-1027

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP al	Infectious and Non-Infectious Diseases of Cattle			
ADP al-3(Rev.)	Brucellosis of cattle	Ames, Iowa	Yes	1-A
		*College Park, Md.	No	
		St. Paul, Minnesota	Yes	1-A
		**Wooster, Ohio	Yes	1-A
		Madison, Wisconsin	Yes	1-A
ADP al-9(Rev.)	Vibriosis of Cattle	Ames, Iowa	Yes	1-B
		Ithaca, New York	Yes	1-B
ADP al-13(R)	Tuberculosis of Cattle	Ames, Iowa	No	
		East Lansing, Mich.	Yes	1-C
ADP al-14(c) (Rev.)	Mucosal-Respiratory Disease-Complex of Cattle	Ames, Iowa	Yes	1-D
		Ft. Collins, Colo.	Yes	1-D
		Lafayette, Indiana	Yes	1-D
		*Ames, Iowa (Univ.)	Yes	1-D
	Bovine Virus Diarrhea	**Ames, Iowa (Univ.)	No	
ADP al-15(R)	Mastitis of Cattle	Ames, Iowa	Yes	1-E
		Davis, California	Yes	1-E
ADP al-17	Respiratory Diseases of Cattle (Shipping Fever)	Ames, Iowa	Yes	1-F
ADP al-18	Leptospirosis of Cattle	*Ames, Iowa	No	
ADP al-19	Infertility in Cattle other than by Vibriosis and Trichomoniasis	Ames, Iowa	No	
ADP al-21	Epizootic Bovine Abortion	Ames, Iowa	No	
		Davis, California	Yes	1-G
ADP al-22	Foot Rot (Infectious Podo-dermatitis) of Cattle	Ames, Iowa	No	
ADP al-24	Etiological, cytological and histochemic studies of Pulmonary Adenomatosis in Cattle	**Ames, Iowa	No	
ADP al-25	Immunization against Bovine Leptospirosis	**Ames, Iowa	Yes	1-H
ADP al-26	Chemotherapy in Leptospirosis	**Ames, Iowa	Yes	1-I
ADP al-27	Nature and Immunogenicity of Leptospiral Lipids	**Ames, Iowa	Yes	1-J
ADP al-35	Paratuberculosis of Cattle (Johne's Disease)	Ames, Iowa	Yes	1-K
ADP al-37	Investigations of Pink Eye (Infectious Keratitis) of Cattle	Ames, Iowa	No	
	The Immunizing Effect of Brucella Cell Wall (PL 480 (A10-ADP-6)	Jerusalem, Israel	Yes	1-A

* Completed during reporting period
** Initiated during reporting period

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP a2	Infectious and Noninfectious Diseases of Swine			
ADP a2-8(Rev.)	Studies on the causative agent (or agents), mode of spread, diagnosis, and control of atrophic rhinitis in swine	Ames, Iowa	No	
ADP a2-10(Rev.)	Transmissible Gastroenteritis (TGE)	Ames, Iowa	Yes	2-C
		Davis, California	Yes	2-C
		Lafayette, Indiana	Yes	2-C
ADP a2-13(Rev.)	Pilot field studies to evaluate diagnostic tests, biologic products, and quarantine measures for a hog cholera eradication program	Live Oak, Florida	Yes	2-A-4
ADP a2-15	Erysipelas of swine	Ames, Iowa	Yes	2-D
		Jersey City, N.J.	Yes	2-D
		Pulawy, Poland	Yes	2-D
ADP a2-16	Brucellosis of Swine	Ames, Iowa	Yes	2-E
ADP a2-17(C)	Hog Cholera	Ames, Iowa	Yes	2-A-1,2, 3
		Urbana, Illinois	Yes	2-A-5
		Lincoln, Nebraska	Yes	2-A-5
ADP a2-18	Infectious causes of infertility in swine other than brucellosis and leptospirosis	Ames, Iowa	No	
ADP a2-19	*Abscesses in Swine	Ames, Iowa	No	

*Initiated during reporting year

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes) (No)	Line Project inc. in Area and Subheading
ADP a3	Infectious and Non-Infectious Diseases of Sheep and Goats			
ADP a3-1(Rev.)	Investigations of vibriosis of Sheep	Ames, Iowa	No	
		Fort Collins, Colo.	Yes	3-B
		Bozeman, Montana	Yes	3-B
		Logan, Utah	Yes	3-B
ADP a3-3	Investigations of scrapie of sheep	Compton, England	Yes	3-C
		Edinburgh, Scotland	Yes	3-C
ADP a3-4	Viral Ulcerative Dermatosis of Sheep	Fort Collins, Colo.	Yes	3-D
ADP a3-5	Investigations of bluetongue in sheep - diagnosis, transmission and control	Denver, Colorado	Yes	3-A
		Pullman, Washington	No	
ADP a3-6	Paratuberculosis (Johne's Disease) of Sheep and Goats	Ames, Iowa	No	

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line project inc. in Summary of Progress (Yes)(No)	Line project inc. in Area and Subheading
ADP b6	Miscellaneous Parasites and Parasitic Diseases			
ADP b6-13(c)*	Investigations on the serological diagnosis, transmission, and control of equine piroplasmosis	Beltsville, Maryland Gainesville, Florida Lexington, Kentucky	Yes Yes No	4-A 4-A
	*Contracts initiated during reporting period			

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP a5	Investigations of Infectious and Non-Infectious Diseases of Poultry			
ADP a5-2(Rev.)	Salmonellosis of Poultry	Ames, Iowa	No	
		Athens, Georgia	Yes	5-C
ADP a7-25	Investigations of the Genus Pasteurella	Ames, Iowa	Yes	5-D
		Pulawy, Poland	Yes	5-D
ADP a5-17	Chronic Respiratory Disease Complex in Chickens and Turkeys	Ames, Iowa	Yes	5-B
		Storrs, Conn.	Yes	5-B
		Newark, Delaware	Yes	5-B
		Athens, Georgia	Yes	5-B
		Amherst, Mass.	Yes	5-B
		Ithaca, New York	Yes	5-B
		Raleigh, N. Car.	Yes	5-B
		College Station, Texas	Yes	5-B
		Blacksburg, Va.	Yes	5-B
		St. Paul, Minn.	Yes	5-B
		Hebrew Univ., Israel	No	
ADP a5-18	Newcastle Disease	Athens, Georgia	No	
		Ames, Iowa	Yes	5-F
		Orono, Maine	Yes	5-F
		Madison, Wisconsin	Yes	5-F
		Pulawy, Poland	Yes	5-F
ADP a5-19(C)**	Bluecomb in Turkeys	St. Paul, Minn.	Yes	5-E
ADP a5-20	Ornithosis in Poultry	Davis, Calif.	Yes	5-A
		St. Paul, Minn.	Yes	5-A
		Corvallis, Oregon	Yes	5-A
		College Station, Texas	Yes	5-A
		Ames, Iowa	No	
ADP a5-21	Turkey Airsacculitis	Ames, Iowa	Yes	5-B
		St. Paul, Minn.	Yes	5-B
		Madison, Wisconsin	Yes	5-B
ADP a5-22	Study of Avian Leukosis	**Ithaca, New York	No	
		E.Lansing, Michigan	No	5-G
ADP a5-23*	Infectious Bronchitis in Poultry	Ames, Iowa	No	
	Fowl Plague	Madrid, Spain (E25-ADP-1)	Yes	5-H

*Initiated during reporting year
**Terminated during reporting year

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP a6	Infectious and Non-Infectious Diseases of Fur Animals, Including Rabbits			
ADP a6-5	Enteric Disease Complex of Rabbits	Fontana, California	Yes	6-A
ADP a6-6	Respiratory Disease Complex of Rabbits	Fontana, California	Yes	6-B
ADP a6-7	Field and Laboratory Studies of Diseases of Fur Animals	Pullman, Washington	Yes	6-C
ADP a6-8	Studies on the Persistence and Transmission of viral and rickettsial diseases in helminths associated with diseases of fur animals	Pullman, Washington	Yes	6-D

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes)	(No)	Area and Subheading
ADP a7	Miscellaneous Infectious and Non-infectious Diseases of Animals				
ADP a7-5(R)	Reservoirs, transmission and immunological studies of vesicular stomatitis	Ames, Iowa		No	
ADP a7-7(R)	**Investigation of livestock poisoning by plants, their toxicity for different classes of livestock and methods of treatment and prevention	Logan, Utah Ames, Iowa Sao Paulo, Brazil (Pl 480 Grant) (S3-ADP-5)	Yes Yes No		7-G 7-G
ADP a7-8(R)	**Investigation of the toxicity of herbicides and herbicide-treated plants to livestock	Logan, Utah		No	
ADP a7-12(R)	Use of radioactive isotopes in studying insecticide toxicology in animals	Kerrville, Texas	Yes		7-C
ADP a7-14(R)	Fractionation, purification and characterization of the components of normal and immune sera of animals	Ames, Iowa	Yes		7-A
ADP a7-15	Bloat in ruminants	Ames, Iowa Davis, California College Park, Md. State College, Miss. Ithaca, New York Madison, Wisconsin	Yes Yes Yes Yes Yes Yes		7-K 7-B 7-B 7-B 7-B 7-B
ADP a7-16	Preparedness for Laboratory Assistance in Diagnosis of Foreign Animal Diseases	Greenport, Long Island New York		No	
ADP a7-17	Studies to develop alleviators and diagnostic tests for plant poisoning and methods to avoid harmful residues in animal tissues from ingesting chemically treated plants	Logan, Utah	Yes		7-H
ADP a7-18	Investigations in cattle and sheep of the biochemical effects of agricultural chemicals and control substances	Kerrville, Texas Nacogdoches, Texas	Yes Yes		7-D 7-D
ADP a7-19	Detoxication mechanisms in cattle and sheep	Kerrville, Texas	Yes		7-E
ADP a7-20	Characterization of cytological responses to toxic actions of antiparasitic and other agricultural chemicals in cattle and sheep tissues	Kerrville, Texas	Yes		7-F
ADP a7-21	Susceptibility of wild animals to foot-and-mouth disease	Greenport, Long Island New York		No	

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Line Project Check List - Area 7, continued

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP a7-22	Studies of the incidence and pathology of cancer and other tumors in food-producing animals	Ames, Iowa Ankara, Turkey (PL 480 Grant) (A22-ADP-2)	No	
ADP a7-23	Toxicological and pathological effects of insecticides, herbicides, fungicides, and other agricultural chemicals on livestock and poultry	Kerrville, Texas	Yes	7-C
ADP a7-24	Mycotic Diseases of Domestic Animals	Ames, Iowa	Yes	7-I
ADP a7-25	Investigations of the Genus Pasteurella	Ames, Iowa	Yes	5-D
ADP a7-26	*Biological Changes Associated with Neuropathological Conditions in Animals	Ames, Iowa	Yes	7-J
ADP a7-27	Physiopathological Investigations of the Interrelations between the Respiratory, Circulatory, and Digestive Systems of Animals	Ames, Iowa	Yes	7-K
ADP a7-28	*Proteins and Other Complex Molecules from Animal Disease Agents Derived Primarily from Surface Structures and Extracellular Products	Ames, Iowa	No	
ADP a7-29	*Chemical and Physical Studies on Microbial Antigens	Ames, Iowa	No	
ADP a7-31	*Physiology of Normal Mammalian Cells Grown in Tissue Cultures	Ames, Iowa	No	

* Initiated during reporting year
** Discontinued during reporting year

Line Project Check List - Reporting Year July 1, 1963 to June 30, 1964

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes)(No)	Area and Subheading
ADP a8	Foot-and-Mouth and Other Exotic Diseases of Cattle			
ADP a8-1(Rev.)	*Histopathological investigations of foot-and-mouth and other exotic infectious diseases of cattle	Greenport, Long Island New York	Yes	8-A
ADP a8-2(Rev.)	*Development of fluorescent antibody technique to locate viruses of foot-and-mouth disease and other exotic diseases in tissue cells	"	Yes	8-B
ADP a8-8(Rev.)	Immunological investigations - Studies on foot-and-mouth disease virus	"	Yes	8-C
ADP a8-10(Rev)	Immunological investigations to determine the mechanism of antibody formation using viruses of exotic animal diseases	"	Yes	8-D
ADP a8-11(Rev)	Immune response to various types and sub-types of foot-and-mouth disease virus	"	Yes	8-E
ADP a8-12(Rev)	Development of methods for production of large quantities of foot-and-mouth disease virus by tissue culture methods	"	Yes	8-F
ADP a8-13(Rev)	*Microcinematographic study of cellular reactions to the agents of exotic diseases	"	Yes	8-G
ADP a8-14(Rev)	Establishment and characterization of cell lines and cell strains for the propagation of foot-and-mouth and other exotic disease agents of cattle	"	Yes	8-H
ADP a8-17(Rev)	Mechanism of the interaction between foot-and-mouth disease virus molecules and host cells	"	No	
ADP a8-18(Rev)	Investigations of the genetic biochemistry of foot-and-mouth disease virus	"	No	
ADP a8-19(Rev)	Effects of certain chemical and physical environments on foot-mouth-disease virus	"	No	
ADP a8-20(Rev)	Bulk freeze-drying of foot-and-mouth disease virus, vaccines, and antiserums	"	No	
ADP a8-23	*Rinderpest of cattle	"	Yes	8-O

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Line Project Check List - Area 8 continued

Work and Line Project Number	Work and Line Project Titles	Work locations During Past Year	Line project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP a8-24	The Survival and Transmission of foot-and-mouth disease virus in the semen of susceptible species of animals	Greenport, Long Island New York	No	
ADP a8-25	Identification, purification, and chemical and physical characterization of foot-and-mouth disease virus and other exotic animal viruses	"	No	
ADP a8-26	Immuno-chemical investigations of foot-and-mouth disease	"	Yes	8-I
ADP a8-27	Microbiological Investigations - Attenuation of representative types of foot-and-mouth disease virus	"	Yes	8-J
ADP a8-28	Survival and inactivation of foot-and-mouth disease virus in meat and meat by-products	"	Yes	8-K
ADP a8-29	Studies on the biological mechanisms of natural resistance and susceptibility of foot-and-mouth disease virus	"	Yes	8-L
ADP a8-30	Biological alterations of foot-and-mouth disease virus from continued residence in cell cultures	"	Yes	8-M
ADP a8-31	**Morphologic Aspects of Virus-Cell Relationships	"	Yes	8-N
ADP a8-32	**Diagnostic and Immunizing procedures for contagious bovine pleuropneumonia	"	No	
	Studies on foot-and-mouth disease (E3-ADP-2)	Sao Paulo, Brazil (PL 480 Grant)	Yes	8-P
	**Studies of various indigenous types of foot-and-mouth disease virus, and the production of a vaccine for the control of FMD in Turkey (A22-ADP-8)	Etlik, Turkey (PL 480 Grant)	No	

* Completed during reporting period
** Initiated during reporting period

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Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP 9	Foot-and-Mouth and Other Exotic Diseases of Swine			
ADP a9-1(Rev.)	Immunological investigations of foot-and-mouth disease of swine	Greenport, L.I. New York	Yes	9-A
ADP a9-2(Rev.)	Investigations of African Swine Fever	Greenport, L. I. New York	Yes	9-B
		Kenya, East Africa	Yes	9-B
		Madrid, Spain	Yes	9-B
ADP 9-3	*Rinderpest in Pigs	Greenport, L. I. New York	Yes	9-C

*Discontinued during reporting year

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Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line project inc. in Summary of Progress (Yes) (No)	Line project inc. in Area and Subheading
ADP all	Foot-and-Mouth and Other Exotic Diseases of Sheep			
ADP all-1	Immunological Investigations of Foot-and-Mouth Disease in sheep	Greenport, L. I., New York	Yes	10-A
ADP all-2	Rinderpest in sheep	"	Yes	10-B

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Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line project inc. in Summary of Progress (Yes)	(No)	Area and Subheading
ADP b1	Parasites and Parasitic Diseases of Cattle				
ADP b1-6(R)*	Etiological factors influencing gastro-intestinal nematodes of cattle	Auburn, Alabama Experiment, Georgia	Yes Yes		11-A 11-A
ADP b1-12(R)	Effects of pasture mixtures and pasture on control of internal parasites	Auburn, Alabama Experiment, Georgia	No No		
ADP b1-19(R)	Acquisition and effects of round-worm parasites of cattle as influenced by diet	Beltsville, Maryland	Yes		11-B
ADP b1-22*	Cultural characteristics and artificial propagation of protozoan parasites	Beltsville, Maryland	Yes		11-C
ADP b1-23(R)	Host-parasite relationship of coccidial parasites of cattle	Auburn, Alabama	Yes		11-D
ADP b1-24	Ecology and immunology of the cattle lungworm, Dictyocaulus viviparus	Beltsville, Maryland	Yes		11-E
ADP b1-25	Clinical and physiological aspects of roundworm parasitism in cattle including anthelmintic treatment	Davis, California	Yes		11-F
ADP b1-26	Investigations of Trichomonad parasites	Logan, Utah Logan, Utah (Univ.)	Yes Yes		11-G 11-G
ADP b1-27	Host-parasite relationship of intestinal worms, Cooperia species in cattle	Auburn, Alabama	Yes		11-H
ADP b1-28	Epizootiological-ecological investigations of the internal parasites of grazing cattle	Beltsville, Maryland	Yes		11-I
ADP b1-29	Etiology and immune response of cattle to winter coccidiosis	Logan, Utah Bozeman, Montana	Yes Yes		11-J 11-J
ADP b1-30	Anaplasmosis of cattle	Beltsville, Maryland Kerrville, Texas	Yes Yes		11-K 11-K
ADP b1-31	Interrelationship of diet and parasitic infection in the production of cattle	Auburn, Alabama	Yes		11-L
ADP b1-32	Histochemistry of gastrointestinal nematodes of cattle	Auburn, Alabama	Yes		11-M
ADP b1-33	Parasites of cattle - with emphasis on Stephanofilarial species	University Park, New Mexico	Yes		11-N

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Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line project inc. in Summary of Progress (Yes)	(No)	Area and Subheading
	Environmental factors influencing parasites and parasitic diseases of economical importance in ruminants (cattle, sheep, and alpacas) (S8-ADP-1)	Lima, Peru	Yes		11-O
	Anaplasmosis, piroplasmosis, and Babesiellosis of cattle (S9-ADP-1)	Montevideo, Uruguay	Yes		11-P
	*Completed during reporting year				

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Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP b2	Parasites and parasitic diseases of swine			
ADP b2-4(Rev.)	The effect of anthelmintic treatment on rate of gain when administered to parasitized pigs of different ages and on different nutrition levels	Tifton, Georgia	Yes	12-C
ADP b2-10(Rev.)	Investigation of the bionomics and pathogenicity of the swine whipworm	Beltsville, Maryland	Yes	12-D
ADP b2-11(Rev.)	Control of swine kidney worms by herd management, etc.	Tifton, Georgia Raleigh, North Carolina	Yes Yes	12-A 12-A
ADP b2-12(Rev.)	Investigations of the swine intestinal roundworm, Ascaris suum	Lincoln, Nebraska	Yes	12-C
ADP b2-15	Investigations of strains of Trichinella spiralis resistant to heat and cold and modes of transmission of the parasite	Beltsville, Maryland	Yes	12-E
ADP b2-17	Studies of Strongyloides ransomi infections in baby pigs	Tifton, Georgia	Yes	12-B
ADP b2-18	Evaluation of biochemical and other aspects of the host-parasite relationship in the development and severity of helminthiases of swine	Beltsville, Maryland	No	
	Investigations on trichinellosis with special reference to epidemiology, immunological diagnosis and pathogenesis (E21-ADP-9)	Warsaw, Poland	Yes	12-E

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Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes)(No)	Area and Subheading
ADP b3	Parasites and Parasitic Diseases of Sheep and Goats			
ADP b3-14	*Investigations of the bionomics of coccidial parasites of sheep and goats	Beltsville, Maryland	Yes	13-A
ADP b3-15	Investigations on the effects of helminthic infections on serum proteins of sheep and goats	Beltsville, Maryland	No	
ADP b3-16	Investigations of gastrointestinal nematodes and nematodiases of sheep and goats and measures for their control	Beltsville, Maryland Lexington, Kentucky	Yes No	15-A
ADP b3-17	The biology of the liver fluke, Fasciola hepatica, of sheep and cattle, etc.	University Park, New Mexico Ankara, Turkey (A22-ADP-1)	Yes No	13-D
ADP b3-18	The life histories, biology, pathogenesis and control of several helminth parasites of sheep occurring in the Southwest	University Park, New Mexico	Yes	13-D
ADP b3-19	Studies on the life cycles of Eimeria ahasta and Eimeria crandallis, pathogenic coccidia of sheep	Auburn, Alabama	Yes	13-B
ADP b3-20	**The effect of gastrointestinal nematodes on the tensile strength and sulfur content of wool	Fargo, North Dakota	Yes	13-E
ADP b3-21	**Immunity to the intestinal worm, Trichostrongylus colubriformis, a parasite of ruminants	Auburn, Alabama	Yes	13-B
ADP b3-22	**Control of the common sheep scab mite, Psoroptes ovis	Albuquerque, New Mexico	Yes	13-C

* Discontinued during reporting year
** Initiated during reporting year

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Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP b4	Parasites and Parasitic Diseases of Poultry			
ADP b4-9	Investigations for Controlling Coccidiosis of Poultry	Beltsville, Maryland	No	
ADP b4-10	The Biology of the Nematode Parasite of Poultry and related birds with Special Reference to the Application of Findings to Control Measures	Beltsville, Maryland	Yes	14-A
ADP b4-11	Biological Investigations of Protozoan Parasites and Parasitic Diseases of Poultry, with Special Reference to those of the Gastrointestinal Tract	Beltsville, Maryland	Yes	14-B

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Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes))No)	Area and Subheading
ADP b5	Treatments for Removal or Control of Parasites of Domestic Animals			
ADP b5-5(Rev.)	Evaluation, development, and standardization of chemical methods of established or reported value for the control of parasitic diseases of livestock and poultry	Beltsville, Maryland Auburn, Alabama	Yes Yes	15-A 15-A
ADP b5-9(Rev.)	Investigations for Treatments for bovine venereal trichomoniasis	Beltsville, Maryland	Yes	15-B
ADP b5-12	Investigations of parasitic and related skin diseases of cattle, sheep, and swine, with primary emphasis on chemical control and basic biology of mange and scabies	Albuquerque, New Mexico	Yes	15-C
ADP b5-13	Pathobiology of parasitic infections with special reference to the injuriousness of arthropod parasites, and the economic gain and efficiency of control measures	Albuquerque, New Mexico	Yes	15-F
ADP b5-14	Development of new methods for the control and eradication of ticks of domestic animals, with special reference to the cattle fever ticks, Boophilus annulatus and B. microplus, the principal vectors of bovine piroplasmosis	Albuquerque, New Mexico	Yes	15-G
ADP b5-15	Development of new approaches and methods for the control and eradication of scabies in sheep and cattle	Albuquerque, New Mexico	Yes	15-E
ADP b5-16	Control of internal parasites of livestock by management practices that will not create consumer residue hazards	Auburn, Alabama	Yes	15-D
ADP b5-17	Investigations of antiparasitic agents and measures for the control of parasites belonging to the family Oestridae	Albuquerque, New Mexico	Yes	15-C
ADP b5-18*	Investigations to develop new and improved chemical agents for the treatment, prevention, or control of helminthic parasites in farm animals	Beltsville, Maryland	Yes	15-C
	* Initiated during reporting period			

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Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line project inc. in Summary of Progress (Yes)	(No)	Area and Subheading
ADP b6	Miscellaneous Parasites and Parasitic Diseases				
ADP b6-2(Rev.)	Identification of parasites of importance in regulatory and other work	Beltsville, Maryland	Yes		16-B
ADP b6-5(Rev.)	Maintenance of author, subject, and host catalogues, etc.	"	Yes		16-A
ADP b6-6(Rev.)	Maintenance of parasite collections	"	Yes		16-D
ADP b6-9(Rev.)	Publication of author, subject (parasite) and host index-catalogues of medical and veterinary zoology	"	Yes		16-A
ADP b6-10	Investigation of immunologic and other biologic approaches to the prevention and control of parasitic diseases	"	Yes		16-B
ADP b6-11	Studies of the chemical and physical elements of parasites and parasite-host relationships in animals	"	Yes		16-C
ADP b6-12	Taxonomic Investigations of Helminths and Other Parasites	"	Yes		16-D